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CHEMICAL ANALYSES FOR WATER QUALITY

TRAINING MANUAL

ENVIRONMENTAL PROTECTION AGENCY

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CHEMICAL ANALYSES FOR WATER QUALITY



TRAINING MANUAL

U.S. ENVIRONMENTAL PROTECTION AGENCY
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This training manual contains outlined information used by lecturers when presenting topics in EPA-NTC Course 100, "Chemical Analyses for Water Quality." The contents concern individual constituents found in water (e.g. dissolved oxygen), instrumental methodology (e.g., atomic absorption) and information useful to analysts (e.g., statistics). A section of laboratory procedures used by class groups is also included.			
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CHEMICAL ANALYSES FOR WATER QUALITY

This course is designed for those persons who either perform chemical analyses for water quality or interpret chemical data, and who have an understanding of basic chemistry and chemistry laboratory procedures.

Learning objectives for the student are:

- To understand selected tests employed in chemical analysis
- To know the advantages and limitations of specific test methods or instrumentation
- To select the more effective analytical methods
- To perform selected wet chemical and instrumental methods, and
- To evaluate results in terms of statistical inferences

Selected analytical procedures are presented in lecture-discussion sessions in the classroom outlining essentials of the tests, control techniques and interpretations. Various methods are stressed to increase specificity and validity of chemical criteria related to water pollution control, and compliance with water quality standards.

ENVIRONMENTAL PROTECTION AGENCY
Water Programs Operations
TRAINING PROGRAM

TRAINING PROGRAM

Through the Water Programs Operations Office, Environmental Protection Agency conducts programs of research, technical assistance, enforcement, and technical training for water pollution control.

The objectives of the Training Program are to provide specialized training in the field of water pollution control which will lead to rapid application of new research findings through updating of skills of technical and professional personnel, and to train new employees recruited from other professional or technical areas in the special skills required. Increasing attention is being given to development of special courses providing an overview of the nature, causes, prevention, and control of water pollution.

Scientists, engineers, and recognized authorities from other Agency programs, from other government agencies, universities, and industry supplement the training staff by serving as guest lecturers. Most training is conducted in the form of short-term courses of one or two weeks' duration. Subject matter includes selected practical features of plant operation and design, and water quality evaluation in field and laboratory. Specialized aspects and recent developments of sanitary engineering, chemistry, aquatic biology, microbiology, and field and laboratory techniques not generally available elsewhere, are included.

The primary role and the responsibility of the states in the training of wastewater treatment plant operators are recognized. Technical support of operator-training programs of the states is available through technical consultations in the planning and development of operator-training courses. Guest appearances of instructors from the Environmental Protection Agency, and the loan of instructional materials such as lesson plans and visual training aids, may be available through special arrangement. These training aids, including reference training manuals, may be reproduced freely by the states for their own training programs. Special categories of training for personnel engaged in treatment plant operations may be developed and made available to the states for their own further production and presentation.

A bulletin of courses is prepared and distributed periodically by the National Training Center. The bulletin includes descriptions of courses, schedules, application blanks, and other appropriate information. Organizations and interested individuals not on the mailing list should request a copy from The National Training Center.

FOREWORD

These manuals are prepared for reference use of students enrolled in scheduled training courses of the Office of Water Programs, Environmental Protection Agency.

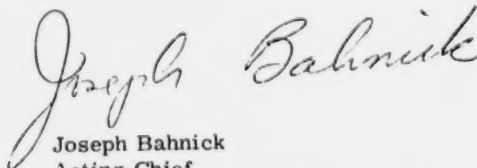
Due to the limited availability of the manuals, it is not appropriate to cite them as technical references in bibliographies or other forms of publication.

References to products and manufacturers are for illustration only; such references do not imply product endorsement by the Office of Water Programs, Environmental Protection Agency.

The reference outlines in this manual have been selected and developed with a goal of providing the student with a fund of the best available current information pertinent to the subject matter of the course. Individual instructors may provide additional material to cover special aspects of their own presentations.

This manual will be useful to anyone who has need for information on the subjects covered. However, it should be understood that the manual will have its greatest value as an adjunct to classroom presentations. The inherent advantages of classroom presentation is in the give-and-take discussions and exchange of information between and among students and the instructional staff.

Constructive suggestions for improvement in the coverage, content, and format of the manual are solicited and will be given full consideration.



Joseph Bahnick
Acting Chief
Direct Technical Training Branch
Division of Manpower and Training
Office of Water Programs
Environmental Protection Agency

U.S. Environmental Protection Agency

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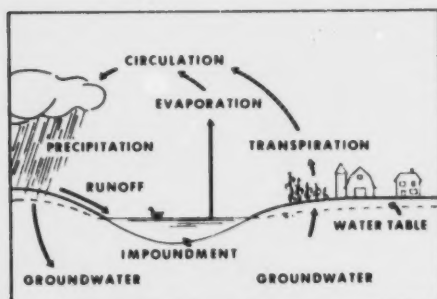
I. WATER RESOURCES, NEEDS AND REQUIREMENTS

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WATER RESOURCES AND NEEDS

I WATER RESOURCES

- A The source of all freshwater is the hydrologic cycle, shown in Figure 1.



THE HYDROLOGIC CYCLE

Figure 1

- 1 Precipitation of water as rain, snow, hail, sleet or dew.
 - 2 Percolation of water through soil to an aquifer to form groundwater.
 - 3 Runoff of water which forms lakes, streams and rivers.
 - 4 Evaporation of surface water or transpiration of water from green plants to the atmosphere.
 - 5 Atmospheric recirculation of the water vapor.
- B The world's supply of water is contained within the hydrologic cycle as:
- 1 Oceanic water
 - 2 Water vapor in the atmosphere
 - 3 Ice and snow in glaciers and snowpack
 - 4 Runoff water in lakes and streams
 - 5 Groundwater

- C Withdrawals for use are mostly from those waters in the runoff and groundwater phases, although some oceanic waters are being utilized.

- D Precipitation--which serves to recharge groundwaters and surface supplies--is at a relatively fixed annual rate.

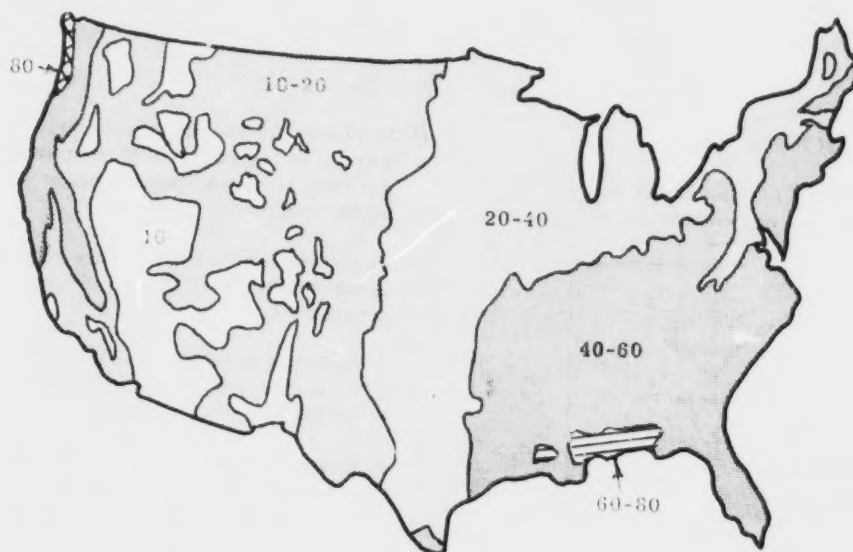
- 1 Average precipitation in the U.S. is 30 inches per year or 4,200-4,400 billion gallons per day.
- 2 Evapo-transpiration losses total approximately 21 inches per year or approximately 2,940 billion gallons per day.
- 3 The available water totals approximately 9 inches per year or 1,260 billion gallons per day.

II THE DISTRIBUTION OF U.S. WATER RESOURCES

Although the water supply in the hydrologic cycle is fixed in amount, it is not distributed evenly. A wide disparity of water distribution exists both in time and space. Distribution of the annual average precipitation is shown in Figure 2.

A Distribution of Precipitation

- 1 Dependent upon:
 - a Atmospheric conditions such as temperature and winds
 - b The geography of the region
 - c The general climate of the area
- 2 U.S. areas of high annual precipitation
 - a The Pacific slope varies from 10 inches to greater than 100 inches annually.



Distribution of Precipitation
(Average Annual)
Inches

Figure 2

- b The gulf states precipitation varies from 20 to 60 inches annually.
 - c Precipitation in the midwest and Great Lakes area ranges from 25 to 50 inches per year.
 - d Precipitation along the Atlantic Coast averages between 35 to 50 inches per year.
- 3 Areas of low annual precipitation
 - a The Rocky Mountain area precipitation ranges between 10 and 20 inches per year.
 - b Much of the southwest has less than 10 inches of precipitation annually.
 - 4 Distribution of precipitation with time
 - a The rainy or wet season varies from summer to winter, or in some areas there is relatively little change a year.
 - b Local storms of high intensity may reach as much as 30 inches in 24 hours.
- B Distribution of Runoff
 - 1 Dependent upon:
 - a Precipitation in the region
 - b Infiltration - which is controlled by the geologic formations and the time lapse between rains.
 - c Season of the year controls evaporation, and snow melt.
 - d Topography controls the time available to percolate through the soil.
 - e Vegetation type and density affects interception and evapotranspiration.
 - 2 Areas of high annual runoff
 - a Sections of the Pacific slope have greater than 80 inches annually.

- b The eastern 1/3 of the U.S. averages greater than 20 inches of runoff annually.
- 3 Much of the western U.S. has less than 1 inch of runoff annually.
 - a Southwest
 - b Rocky Mountain states
 - c Rocky Mountain plateau
- 4 Time distribution of runoff
 - a Overflow--runoff during and immediately following precipitation.
 - b Base flow--sustained or fair weather runoff composed of delayed sub-surface and groundwater runoff. See Figure 3 for runoff cycle.

C Distribution of Groundwater

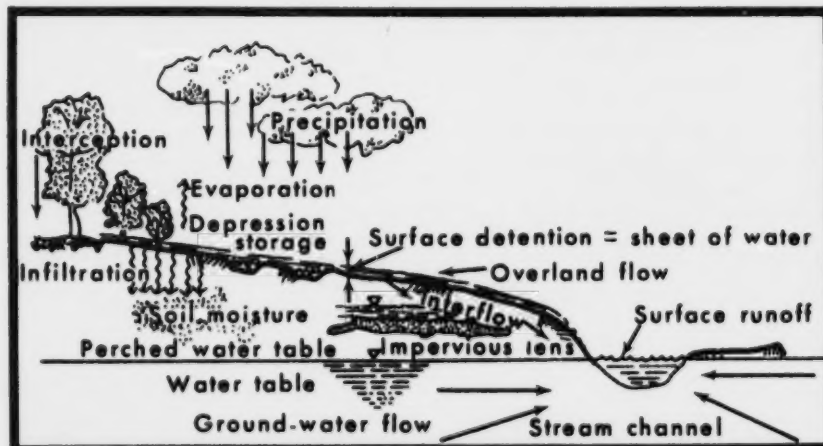
- 1 Groundwater volume is affected by the same factors as runoff.
- 2 Geologic formations and soils control percolation and storage of groundwater.

- 3 Topography controls time available for percolation.
- 4 Evapo-transpiration varies with the season, as does precipitation and ground saturation.

III WATER USE

A Present Water Use in the U.S.

- 1 Water available for use
 - a Nine inches or 1,160 billion gallons per day are not lost through evapo-transpiration, and is therefore theoretically available.
 - b Water use in the U.S. at the present time is approximately 390 billion gallons per day or 3 inches of our total supply.
 - c Twenty-one inches are lost through evapo-transpiration.



THE RUNOFF CYCLE
(Davis & DeWiest)
Figure 3

2 The way in which water is used

Water uses can be grouped into two classes. Those uses which are in situ such as recreation, fishing, and wildlife and those uses requiring withdrawal from the stream. These withdrawals are:

- a Agricultural uses take 46% of our supply or 180 billion gallons/day; only 40% of this water is returned to the streams.
- b Industrial uses take another 46% of our supply. 2% of the water used by industry is consumed.
- c Municipal uses total approximately 25 billion gallons daily or 8% of the total.

3 Source of water used in U.S.

- a National averages show 80% or 312 billion gallons per day to be from surface sources, while 20% is taken from the ground.
- b The ratio of surface water to ground-water varies and is dependent on the quantity and quality available in each locality, as well as the cost.

4 Seasonal uses of water

- a Irrigation waters are used during the growing season only.
- b Some water using industries such as the canning industry are seasonal.
- c The majority of industries needs water throughout the year.
- d Municipal use is higher in the summer.

B Demand for water is increasing

- 1 The predicted demand of water in 1980 is approximately 600 billion gallons of water per day, or 220,000 billion per year.

2 This is mainly due to expansion of industry and irrigated agriculture.

3 Much of the demand for water will be in areas such as the southwest, that are already short on water.

C Methods for the Development of U.S. Water Resources for Future Needs

- 1 Utilization of our present sources of water, surface and groundwater, must be increased. This would mean increased storage, both on the surface and in underground reservoirs.
- 2 Desalinization of ocean waters and brackish waters holds some promise for regions where transportation will not be expensive.
- 3 Reduction of evapo-transpiration losses will greatly increase our total available supply.
- 4 Weather modification methods could possibly give us precipitation in the right place at the right time.
- 5 Greater reuse of our present supply is both through multiple use and better waste treatment methods.

IV SUMMARY

The total amount of water available appears to be fixed. In view of the increasing demands and the currently inefficient utilization of the supply, the demand may very shortly exceed the supply. Better management of the resource and more engineering research are urgently needed.

ACKNOWLEDGEMENT:

Certain portions of this outline contain training material from prior outlines by Peter F. Atkins, F. P. Nixon.

Table 1. AVAILABILITY OF GROUND WATER

Areas	Water Use (excluding water power) Use in mgd and Percent of total from Ground Water Sources	
	Total mgd	Ground water (%)
A Atlantic and Gulf Coastal Plain area	32,000	25
B Southern Great Plains area	21,000	45
C Appalachian Mountain and Piedmont area	8,000	50
D Rocky Mountains, northern Great Plains, and northern Pacific Coast area	28,000	12
E Unglaciated central plateaus and lowlands	26,000	10
F-1 Basin and range	41,000	42
F-2 Columbia Plateau	24,000	7
G Glaciated area of the East and Midwest	57,000	10
U.S. Total (rounded)	240,000	20

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This outline was prepared by Edward D. Schroeder, Engineer, formerly with the National Training Center and revised by L. J. Nielson, Categorical Programs Division, EPA, Region X, 1200 Sixth Avenue, Seattle, WA 98101.

WATER QUALITY CRITERIA

INTRODUCTION

On February 27, 1967, the Secretary of the Interior established the first National Technical Advisory Committee on Water Quality Criteria for the Federal Water Pollution Control Administration (now designated Water Programs Operations). The principal function of this group was to collect into one volume a basic foundation of water quality criteria.

One troublesome problem encountered by the Committee was to distinguish the terms "criteria" and "standard". Regardless of any other uses of the words, the following definitions are considered appropriate:

Standard - A plan that is established by governmental authority as a program for water pollution prevention and abatement.

Criteria - A scientific requirement on which a decision or judgment may be based concerning the suitability of water quality to support a designated use.

The Federal Water Pollution Control Act authorizes the States to set standards. Quality characteristics of a physical, chemical, or biological nature demanded by aquatic life, industrial process, or other use, are requirements or criteria. The criteria are a significant part of water quality standards. The Committee considered the water use criteria set forth in this report with the objective of assisting the State and Federal agencies in setting and evaluating standards so they can meet water pollution abatement objectives.

The Committee subdivided to develop criteria for five general areas of water use:

1. Recreation and Aesthetics
2. Public Water Supplies
3. Fish, Other Aquatic Life and Wildlife
4. Agriculture
5. Industry

Following in this outline of the manual you will find summaries of the reports for each of these five uses. These summaries must be considered an abridgement of the Committee's Report. The original Report should be read in its entirety if the Water Programs Operations Criteria for each use are to be clearly understood.

The statement of Water Quality Criteria is currently being revised so is no longer available from the U. S. Government Printing Office.

Determining water quality criteria for various water uses is an important step in solving the Nation's water pollution problems. Along with vigorous implementation programs, it is a necessary step in achieving water quality management on a scientific basis. The Committee firmly believes that preserving and improving the quality of our water resources is well worth our best efforts.

Achieving water quality goals will depend on people: alert and responsible administrators at all levels of government and industry, well-trained scientists, engineers, and technicians, sympathetic legislators and stockholders, and an informed public.

WATER QUALITY CRITERIA
AESTHETICS AND RECREATION

Summary of Recommendations

I AESTHETICS

A General Requirements

- 1 All surface waters should be capable of supporting life forms of aesthetic value.
- 2 Surface waters should be free of substances attributable to discharges or wastes as follows:
 - a Materials that will settle to form objectionable deposits.
 - b Floating debris, oil, scum, and other matter.
 - c Substances producing objectionable color, odor, taste, or turbidity.
 - d Materials, including radionuclides, in concentrations or combinations which are toxic or which produce undesirable physiological responses in human, fish, and other animal life and plants.
 - e Substances and conditions or combinations thereof in concentrations which produce undesirable aquatic life.

B Desirable Additional Requirements

- 1 The positive aesthetic values of water should be attained through continuous enhancement of water quality.
- 2 The aesthetic values of unique or outstanding waters should be recognized and protected by development of appropriate criteria for each individual case.

II RECREATION

A General Recreational Use of Surface Waters

- 1 Surface waters, with specific and limited exceptions, should be suitable for human use in recreation activities not involving significant risks of ingestion without reference to official designation of recreation as a water use. For this purpose, in addition to aesthetic criteria, surface waters should be maintained in a condition to minimize potential contamination by utilizing fecal coliform criteria for monitoring. In the absence of local epidemiological experience, the subcommittee suggests an average not to exceed 2,000 fecal coliforms per 100 ml and a maximum of 4,000 per 100 ml, except in specified mixing zones adjacent to outfalls.
- 2 Surface waters, with specific and limited exceptions, should be of such quality as to provide for the enjoyment of recreation activities based upon the utilization of fishes, waterfowl, and other forms of life without reference to official designation of use. The Subcommittee recommends by reference criteria developed by the National Technical Advisory Subcommittee on Fish, Other Aquatic Life, and Wildlife for guidance relative to various species and waters.
- 3 Species available for harvest by recreation users should be fit for human consumption. In areas where taking of mollusks is a recreational activity, the criteria shall be guided by the U.S. Public Health Service manual, "Sanitation of Shellfish Growing Areas," 1965 revision.

B Enhancement of Recreation Value of Waters Designated for Recreation Uses Other Than Primary Contact Recreation

- 1 In waters designated for recreation use other than primary contact recreation, the fecal coliform content, as determined by either multiple-tube fermentation or membrane filter techniques, should not exceed a log mean of 1,000/100 ml, nor equal or exceed 2,000/100 ml in more than 10% of the samples.
- 2 In waters designated for recreation use, optimum conditions for recreation based upon utilization of fish, other aquatic life, and wildlife should apply, with specific and limited exceptions. The Subcommittee endorses by reference the criteria for these purposes recommended by the National Technical Advisory Subcommittee on Fish, Other Aquatic Life, and Wildlife.

C Primary Contact Recreation

1 Criteria for mandatory factors

- a Fecal coliform should be used as the indicator organism for evaluating the microbiological suitability of recreation waters. As determined by multiple-tube fermentation or membrane filter procedures and based on a minimum of not less than five samples for any 30-day period of the recreation season, the fecal coliform content of primary contact recreation waters shall not exceed a log mean of 200/100 ml, nor shall more than 10 percent of total samples during any 30-day period exceed 400/100 ml.
- b In primary contact recreation waters, the pH should be within the range of 6.5-8.3 except when due to natural causes and in no case shall be less

than 5.0 nor more than 9.0. When the pH is less than 6.5 or more than 8.3, discharge of substances which further increases unfavorable total acidity or alkalinity should be limited.

2 Criteria for desirable factors

- a For primary contact waters, clarity should be such that a Secchi disc is visible at a minimum depth of 4 feet. In "learn to swim" areas, the clarity should be such that a Secchi disc on the bottom is visible. In diving areas, the clarity shall equal the minimum required by safety standards, depending on the height of the diving platform or board.
- b In primary contact recreation waters, except where caused by natural conditions, maximum water temperature should not exceed 85° F (30° C).

3 Marine waters

The Subcommittee suggests, as a general practice, application of a single set of criteria for fresh, estuarine, and marine waters.

The preceding is an abridgement. For the complete statement of the Office of Water Programs Criteria for Recreation and Aesthetics, see "Water Quality Criteria, Report of the National Technical Advisory Committee," April, 1968.

WATER QUALITY CRITERIA PUBLIC WATER SUPPLIES

I SUPPLY AND DEMAND

- A Ideally, public water should be available in ample quantity and in undefiled quality to satisfy all potential users, whether for domestic, industrial, recreational, or irrigation purposes.
- B Realistically, nature and man's endeavors are not compatible with such a goal.
- 1 Climate severely limits the amount of water available in many areas.
 - 2 When man uses water, he imparts undesirable constituents to it before returning it to the natural environment.
- C In the early history of our country, and even for a good part of this century, many municipalities could withdraw water for public consumption with no treatment, except possibly chlorination.
- D Today, virtually every community has a considerable capital investment in water treatment facilities.

II WATER QUALITY AND WASTE TREATMENT

- A The cost of treating water for public use is directly related to the quality of treatment given our wastewater.

One cycle of municipal use (as measured by the difference in tap water and treatment plant effluent qualities) increases organic and mineral concentrations substantially. See Table 1.

- B In general, wastewater treatment has not kept pace with expanding population.
- 1 The self-purifying capacity of our rivers and lakes has been exceeded.
 - 2 Many new exotic and harmful compounds, a product of our scientific age, are not removed either by waste or water treatment.

- 3 As late as 1968, only 43% of our wastewater received secondary treatment. Only 65% received any kind of treatment (See Tables 2 & 3 below).
- 4 As an illustration of how the burden has been placed on the rivers, there currently are still no secondary treatment plants on the Ohio or Mississippi Rivers. This situation, fortunately, will end by 1975.
- 5 The breakdown of the Nation's waste treatment facilities is given in Table 4. Trickling filters and stabilization ponds represent about half of the total waste treatment plants in this country.

III WATER TREATMENT TECHNOLOGY

- A The conventional water treatment plant employs techniques which primarily clarify the water and render it hygienically safe. Depending on the source, it may also be necessary to:
- 1 Soften the water by adding lime and sodium carbonate.
 - 2 Employ activated carbon to improve taste and odor.
- B The individual treatment units generally consist of a flash mixing chamber followed in sequence by a flocculation basin, sedimentation tank, rapid sand filtration system, and a chlorine contact chamber. Prechlorination also is sometimes practiced. A typical plant is pictured schematically in Figure 1.
- C Coagulants utilized most commonly are alum, various iron compounds, or lime. The effectiveness of the coagulation step is highly dependent on pH. Small doses of polymers will aid coagulation, but very few polymers have been approved for use in water treatment.

TABLE 1.-Water Quality Depreciation by One Cycle of Municipal Use

Test	Increments in mg/l						Increments in lb/day/1,000 population					
	Batavia	Dayton	Hamilton	Lebanon	Loveland	Average	Batavia	Dayton	Hamilton	Lebanon	Loveland	Average
COD--unfiltered	163	163	163	81	141	143	95	207	188	98	110	140
COD--filtered	120	108	90	70	87	95	70	137	104	82	68	92
COD--filtered, corrected for Cl ⁻	112	98	79	46	76	82	65	124	91	54	59	79
Anionic detergents (ABS)	10.1	7.2	6.2	4.6	9.0	7.4	5.0	9.1	7.1	5.4	7.0	6.9
Hydroxylated aromatic (tannic acid)	2.9	1.3	2.0	0.5	1.4	1.6	1.7	1.7	2.3	0.6	1.1	1.5
Carbohydrates (glucose)	2.6	2.1	5.0	0.9	1.5	2.4	1.5	2.7	5.8	1.1	1.2	2.5
Organic nitrogen (N)	4.0	1.4	3.5	1.0	1.4	2.2	2.3	1.8	4.0	1.2	1.1	2.1
Nitrate (N)	3.1	3.5	1.0*	7.7	4.0	3.5	1.8	4.5	1.2*	9.0	3.1	3.4
Nitrite (N)	0.58	0.10	0.04	0.25	0.55	0.30	0.34	0.13	0.05	0.29	0.43	0.25
Ammonia (N)	6.8	16.3	22.1	19.4	15.7	16.1	3.9	20.7	23.4	22.7	12.3	17.0
Total nitrogen (N)	14.5	21.3	24.6	28.4	21.6	22.0	8.4	27.1	28.3	33.2	16.9	22.8
Total alkalinity (CaCO ₃)	152	160	217	40	41	122	88	203	250	47	32	124
Calcium (Ca ⁺⁺)	43	31	42	1	1	23	25	39	48	1	0.8	24
Magnesium (Mg ⁺⁺)	9	10	11	3	1	7	5	13	13	4	0.8	7
Potassium (K ⁺)	11.7	7.2	9.2	8.2	10.0	9.3	6.8	9.1	10.6	9.6	7.8	8.8
Sodium (Na ⁺)	42	50	46	98	46	57	21	64	53	115	36	58
Phosphate (PO ₄ ⁼)												
Total	34.6	19.4	19.5	18.5	29.4	24.3	20.1	24.6	22.4	21.7	22.9	22.3
Ortho	33.8	17.9	18.9	15.6	27.5	22.8	19.6	22.7	21.7	18.3	21.5	20.8
Sulfate (SO ₄ ⁼)	39	12	52	15	46	33	23	15	60	18	36	30
Chloride (Cl ⁻)	40	51	40	102	46	56	23	65	46	119	36	58
Residue 105°C	374	348	457	128	148	291	217	442	526	150	115	290
Residue 600°C	281	231	312	158	127	222	163	293	350	185	99	220
Loss on ignition	33	117	145	30**	21	60	54	140	167	35**	16	70

* Loss.

** Gain.

Note: Lb x 0.454 = kg.

Table 2

POPULATION WITH WASTE SYSTEMS FOR
YEAR 1968

200 X 10 ⁶	Total population
140 X 10 ⁶	Total sewered (70%)
130 X 10 ⁶	Receive treatment
	93% of sewered
	65% of total

Table 3

TREATMENT CLASSIFICATION BY POPULATION
FOR YEAR 1968

Population	Type of Treatment	% of Treated	% of Total
43 X 10 ⁶	Primary-intermediate	33	22
41 X 10 ⁶	Activated sludge	31	21
28 X 10 ⁶	Trickling filter	22	14
6 X 10 ³	Stabilization pond	5	3
12 X 10 ⁶	Other	9	6
10 X 10 ⁶	Raw discharge	-	5

Table

Table 4

TREATMENT CLASSIFICATION BY FACILITIES
FOR YEAR 1962

Number	Type	% of Total
14,123	Total	100.
1,558	Raw discharge	11.
12,565	Treated discharge	89.
2,459	Primary-intermediate	17.
9,951	Secondary	70.
2,110	Activated sludge (21% of secondary)	15.
3,786	Trickling filters (38% of secondary)	27
3,457	Stabilization ponds (35% of secondary)	25

D Detention times and/or hydraulic loading rates for the various units range as follows:

- 1 Flash mix - 10 to 15 sec.
 - 2 Flocculation - 30 to 60 min. (longer for lime-soda softening).
 - 3 Sedimentation - 2 to 6 hours with preference for smaller period. Overflow rates vary from 500 to 1,500 gal/sq. ft/day.
 - 4 Rapid sand filtration - 2 to 3 gal/sq. ft/min. with suspended solids of applied water no more than 10 mg/l, preferably 5 mg/l.
 - 5 Chlorination - at least 30 minute contact before consumption.
- E Solids - contact or upflow clarifiers are being increasingly employed to combine mixing, flocculation, and clarification in one unit. Space is conserved and initial cost is usually lower. Higher loading rates, 1 hr detention and 1 gal/sq ft/min overflow rate, are possible but rapid variations in raw water turbidity have a greater effect on product quality.
- F Dissolved solids and trace elements and compounds are little affected by the above conventional treatment sequence. We rely on dilution to lower the concentration of these elements below the toxic level. Calcium, magnesium, and phosphorus can be removed by precipitation and iron and manganese by aeration but ion exchange and other tertiary processes are needed to remove most other ions.
- G Direct reuse of wastewater is a possible consideration for some areas in the not-too-distant future. Treatment requirements for direct reuse include secondary wastewater treatment plus carbon adsorption plus nitrogen removal plus demineralization plus breakpoint chlorination for destruction of viruses and bacterial pathogens.

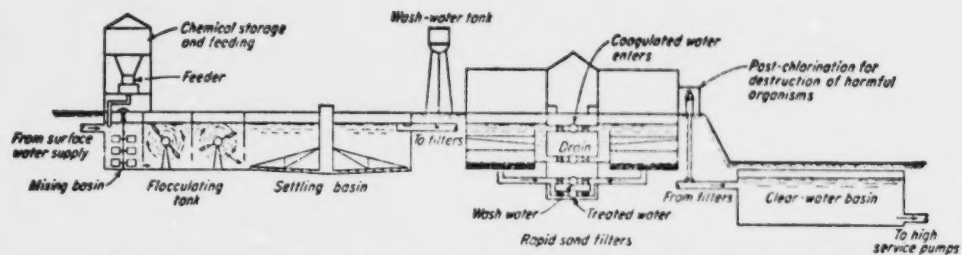
IV STANDARDS FOR DRINKING WATER AND PUBLIC WATER SUPPLIES

- A The Public Health Service is charged with setting standards for acceptable drinking water for the Nation. The latest set of standards were published in 1962. A revised set of standards will probably be forthcoming in the not-too-distant future.
- B The Drinking Water Standards are concerned with bacteriological quality, physical and chemical characteristics, and radioactivity.
- C Sampling frequency is most critical in determining bacteriological quality. Figure 2 indicates the minimum number of samples per month based on population served recommended by Public Health Service. They range from two samples per month for 1,000 people up to 500 per month for 10,000,000 people.
- D The Public Health Service 1962 Drinking Water Standards are summarized in Table 5.
- E In 1968, the National Technical Advisory Committee on Water Quality Criteria formally reported to the Secretary of the Interior on five aspects affecting water use in this country. The five were recreation and aesthetics, public water supplies, fish and other aquatic life, agricultural uses, and industrial water supplies.
- F The Subcommittee for Public Water Supplies developed raw water quality criteria for public water supplies based on reasonable treatment effort which would yield saleable water meeting the Drinking Water Standards.
- 1 Reasonable treatment is limited by the subcommittee to coagulation and flocculation with no more than 50 mg/l alum or iron coagulants plus alkali (but no coagulant aids or activated carbon), sedimentation (six hours or less), rapid sand filtration (three gal/sq. ft/min. or less), and chlorination (without regard to form or concentration of residual).
 - 2 Permissible criteria define acceptable characteristics and concentrations of substances in raw surface waters which allow the production of a water meeting the limits of the Public Health Service Drinking Water Standards without employing any more than the above treatment.

Table 5
USPHS
Drinking Water Standards
1962

Constituent	Recommended Limit	Maximum Permissible Limit
A. Bacteriological		
Requirements vary depending on number of samples collected per month and on type of test.		
In general:		
1. Positive fermentation based on MPN technique		
a. 10 ml standard portions	≤ 10% positive/mo.	
b. 100 ml standard portions	≤ 60% positive/mo.	
2. Membrane filter	≤ 1/100 ml	
B. Physical		
Turbidity (Jackson units)	5	
Color (Color units platinum-cobalt standard)	15	
Threshold Odor Number	3	
C. Chemical (mg/l)		
Alkyl Benzene Sulfonate (ABS)	0.5	
Arsenic (As)	0.01	0.05
Barium (Ba)		1.0
Cadmium (Cd)		0.01
Carbon Chloroform Extract (ccE)	0.2	
Chloride (Cl)	250.	
Chromium (Hexavalent) (Cr ⁺⁶)		0.05
Copper (Cu)	1.0	
Cyanide (CN)	0.01	0.2
Fluoride (F) - temperature dependent	0.8 - 1.7	
Iron (Fe)	0.3	
Lead (Pb)		0.05
Nitrate (NO ₃)	45.0	
Phenols	0.001	
Selenium (Se)		0.01
Silver (Ag)		0.05
Sulfate (SO ₄)	250.0	
Total Dissolved Solids (TDS)	500.0	
Zinc (Zn)	5.0	
D. Radioactive (pc/l)		
Radium - 226	3	
Strontium - 90	10	
Gross beta	1,000	

Figure 1

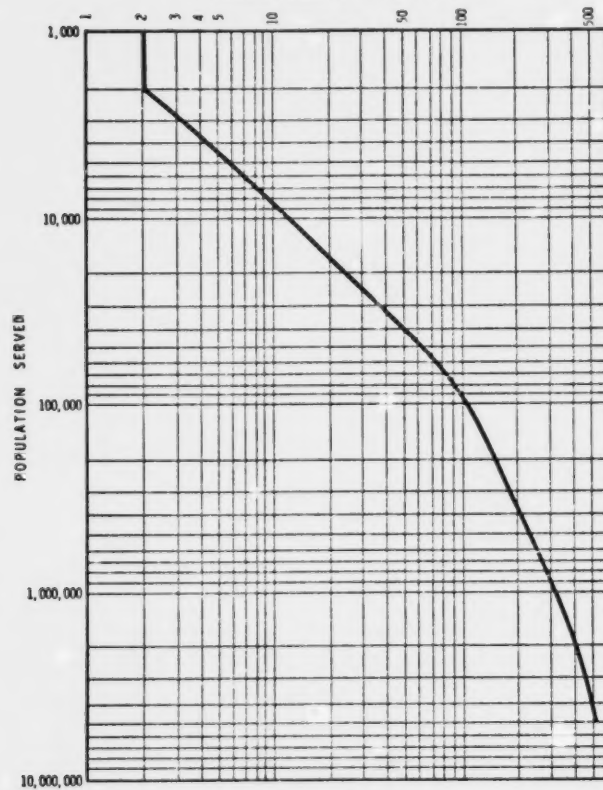


Filtration plant, including coagulation, settling, filtration, postchlorination and clear-water storage. Prechlorination, i.e., addition of chlorine at the mixing basin, is also common practice.

Figure 2

DRINKING WATER STANDARDS, 1962

MINIMUM NUMBER OF SAMPLES PER MONTH



Report of the
National Technical Advisory Committee
on Water Quality Criteria
to the Secretary of the Interior

TABLE 6. Surface Water Criteria for Public Water Supplies

Constituent or characteristic	Permissible criteria	Desirable criteria	Paragraph
Physical:			
Color (color units)	75	<10	1
Odor	Narrative	Virtually absent	2
Temperature *	do	Narrative	3
Turbidity	do	Virtually absent	4
Microbiological:			
Coliform organisms	10,000/100 ml ¹	<100/100 ml ¹	5
Fecal coliforms	2,000/100 ml ¹	<20/100 ml ¹	5
Inorganic chemicals:	(mg/l)	(mg/l)	
Alkalinity	Narrative	Narrative	6
Ammonia	0.5 (as N)	<0.01	7
Arsenic *	0.05	Absent	8
Barium *	1.0	do	8
Boron *	1.0	do	9
Cadmium *	0.01	do	8
Chloride *	250	<25	8
Chromium, * hexavalent	0.05	Absent	8
Copper *	1.0	Virtually absent	8
Dissolved oxygen	>4 (monthly mean) >3 (individual sample)	Near saturation	10
Fluoride *	Narrative	Narrative	11
Hardness *	do	do	12
Iron (filterable)	0.3	Virtually absent	8
Lead *	0.05	Absent	8
Manganese * (filterable)	0.05	do	8
Nitrates plus nitrites *	10 (as N)	Virtually absent	13
pH (range)	6.0-8.5	Narrative	14
Phosphorus *	Narrative	do	15
Selenium *	0.01	Absent	8
Silver *	0.05	do	8
Sulfate *	250	<50	8
Total dissolved solids * (filterable residue)	500	<200	16
Uranyl ion *	5	Absent	17
Zinc *	5	Virtually absent	8
Organic chemicals:			
Carbon chloroform extract * (CCE)	0.15	<0.04	18
Cyanide *	0.20	Absent	8
Methylene blue active substances *	0.5	Virtually absent	19
Oil and grease *	Virtually absent	Absent	20
Pesticides:			
Aldrin *	0.017	do	21
Chlordane *	0.003	do	21
DDT *	0.042	do	21
Dieldrin *	0.017	do	21
Endrin *	0.001	do	21
Heptachlor *	0.018	do	21
Heptachlor epoxide *	0.018	do	21
Lindane *	0.056	do	21
Methoxychlor *	0.035	do	21
Organic phosphates plus carbamates *	0.1 ¹	do	21
Toxaphene *	0.005	do	8
Herbicides:			
2,4-D plus 2,4,5-T, plus 2,4,5-TP *	0.1	do	21
Phenols *	0.001	do	8
Radioactivity:	(pc/l)	(pc/l)	
Gross beta *	1,000	<100	8
Radium 226 *	3	<1	8
Strontium 90 *	10	<2	8

* The defined treatment process has little effect on this constituent.

¹ Microbiological limits are monthly arithmetic averages based upon an adequate number of samples. Total coliform

limit may be relaxed if fecal coliform concentration does not exceed the specified limit.

As parathion is cholinesterase inhibition, it may be necessary to resort to even lower concentrations for some compounds or mixtures. See par. 21.

3 Desirable criteria define characteristics and concentrations of a truly high quality surface water supply which can be processed with a greater safety factor by the above treatment to meet the Standards.

4 Both sets of criteria are given in Table 6. Where the term narrative appears, the Subcommittee could not arrive at a single numerical value which would be applicable throughout the country for all conditions.

G An examination of the Subcommittee's recommendations reveals that they are more encompassing than the Drinking Water Standards. Undoubtedly, the next issue of the Drinking Water Standards will take cognizance of the Subcommittee's Report.

V WATER CONSTITUENTS

The significance of constituents with defined limits in the Drinking Water Standards and the Subcommittee's report are discussed briefly.

A Total Coliforms

- 1 Considered reliable indicators as to possible presence of bacterial pathogens.
- 2 In general, all coliform organisms exhibit similar survival and resistance patterns.
- 3 Presence of any type of coliform in treated drinking water indicates inadequate treatment or excess of contamination after treatment.

B Fecal Coliforms

- 1 Considered only in Subcommittee's Report.
- 2 Indigenous to intestinal tract of warmblooded animals.
- 3 Presence indicates recent fecal pollution.

C Physical Characteristics

- 1 Turbidity, color, and odor limits defined in PHS Standards.
- 2 Subcommittee's Report includes above three plus temperature.

3 Physical characteristics related to consumer acceptance rather than safety of the water.

4 PHS limits are those concentrations at which the physical characteristics become objectionable to the senses.

5 Well operated treatment plants should do much better.

D ABS

- 1 Anionic surfactant.
- 2 Above 1.5 mg/l, taste, odor and foaming complaints are common.
- 3 The recommended limit of 0.5 mg/l is 15,000 times less than the sub-acute toxic level to rats.

E Arsenic

- 1 Cumulative effect.
- 2 Absorbed into body tissues and fluids.
- 3 Suspected of being carcinogenic.

F Barium

- 1 Recognized as a general muscle stimulant.
- 2 Capable of causing nerve block and transient increases in blood pressure.
- 3 Acute toxicity associated with ingestion of barium salts such as chloride due to irreversible changes in tissues.

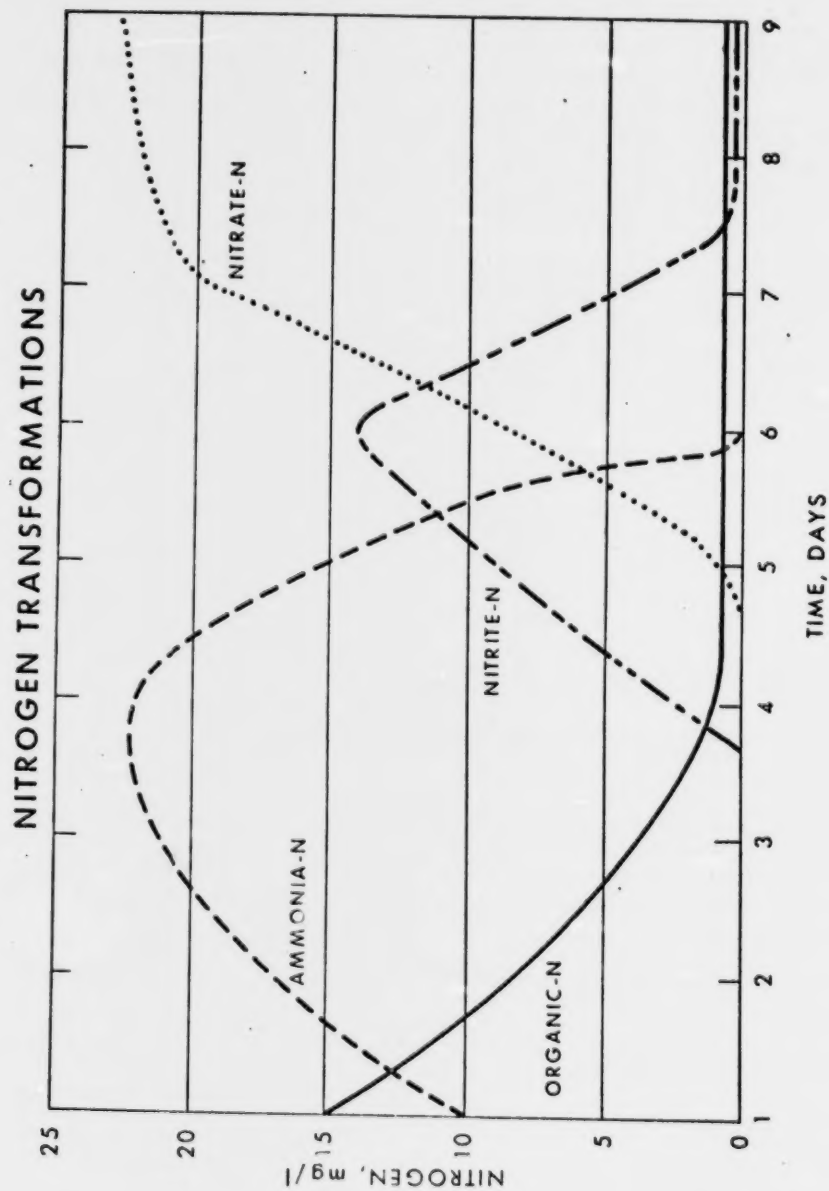
G Cadmium

- 1 Nonessential, nonbeneficial element to biological processes of man.
- 2 Possesses high toxic potential.
- 3 Studies have shown accumulation in soft tissues and marked anemia and retarded growth in rats and adverse renal arterial damage to man.

H Carbon Chloroform Extract

- 1 Indicates man-made or natural organic pollutants not removed in water treatment.
- 2 High sensitivity.

Figure 3



- 3 Difficult to identify exact chemical and toxicological nature of extracted material.

I Chloride, Sulfate and Dissolved Solids

- 1 Importance associated with taste and laxative properties.
- 2 Laxative effect of sulfates particularly noticeable to newcomers and casual users.
- 3 Experience has shown consumer shifts to other supplies (such as bottled water) when mineral concentrations become objectionable.

J Chromium

- 1 Tolerance when ingested not known at present.
- 2 When inhaled, chromium is a known carcinogenic agent for man.
- 3 Toxicity associated only with hexavalent form.
- 4 Limit of Cr^{+6} at 0.05 mg/l based on analytical sensitivity.

K Copper

- 1 Essential in human metabolism.
- 2 Does impart some taste to water above 1 mg/l.
- 3 Large doses may result in liver damage.
- 4 Does not constitute a health hazard at low levels.

L Cyanide

- 1 Standard based more on toxicity to fish than to man.
- 2 Cyanide readily detoxified to thiocyanate in the body.
- 3 Cyanide reduced to levels of about 0.01 mg/l with chlorination under alkaline conditions.

M Fluoride

- 1 Valuable oral decay preventative in children at proper doses.

- 2 Only harmful effect documented in U. S. A. is mottling of teeth at excessive concentrations.

N Iron and Manganese

- 1 Objectionable because of astringent taste and brownish color imparted in excessive amounts.
- 2 No health considerations.

O Lead

- 1 Acutely toxic to humans.
- 2 Normal constituent in many food sources and cigarettes.
- 3 Bacterial decomposition of organic matter inhibited by concentrations above 0.1 mg/l.

P Nitrate and Nitrite

- 1 Nitrite can cause fatal poisoning due to a condition known as methemoglobinemia.
- 2 With infants, nitrates can result in the same situation because the low acidity of the infant's stomach allows certain bacteria to reduce nitrate to nitrite.
- 3 The normal adult's stomach is too acid for this to occur.
- 4 Other detrimental effects attributable to nitrogen compounds (including ammonia) are shown in Table 7.
- 5 The transformation of nitrogen compounds when an inoculum is aerated for several days is indicated in Figure 3. The total amount of nitrogen present is constant throughout the transformation.

Q Phenols

- 1 Concentrations injurious to health far greater than those which impart unpleasant taste or affect fish.

R Selenium

- 1 Produces "alkali disease" in cattle.
- 2 Potential carcinogen to man.

TABLE 7. Importance of Nitrogen

NH₃ in effluents can cause DO sag in receiving water

NH₃ is corrosive to copper fittings

1 NH₃ requires 7 plus Cl₂ for breakpoint

NO₂ causes high Cl₂ demand

NH₃ influences Cl₂ contact time

Nitrogen compounds are nutrients

NO₃ can be health hazard

S Silver

- 1 Sometimes added to water for disinfection.
- 2 Chief effect on body is cosmetic, producing a blue-grey discoloration of eyes, skin, and mucous membranes.

T Zinc

- 1 Esthetic deterrent mainly due to acute, but transitory, gastro-intestinal irritation.

U Radioactive Materials

- 1 Radium-226, Strontium-90, and Gross beta are the three forms of radioactive substances limited by PHS.
- 2 Limits based on recommendations of Federal Radiation Council and approved by the President.

V Additional Substances Considered by Subcommittee Report, but not by PHS Standards

- 1 Alkalinity
- 2 Ammonia
- 3 Boron
- 4 Dissolved Oxygen

5 Hardness

6 pH

7 Phosphorus

8 Uranyl Ion

9 Oil and Grease

10 Pesticides

11 Herbicides

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This outline was prepared by Richard C. Brenner, Research Sanitary Engineer, Advanced Waste Treatment Research Laboratory, Environmental Protection Agency, Office of Water Programs, Cincinnati, Ohio 45268.

WATER QUALITY CRITERIA
FISH, OTHER AQUATIC LIFE, AND WILDLIFE
Summary and Key Criteria

RECOMMENDATIONS given below are considered to be satisfactory for aquatic life. In all instances where natural conditions fall outside the recommended ranges, these conditions may be marginal and should not be changed in such a way as to make them more unfavorable.

Freshwater organisms

Dissolved Materials

(1) Dissolved materials that are relatively innocuous; i.e., their harmful effect is due to osmotic effects at high concentrations, should not be increased by more than one-third of the concentration that is characteristic of the natural condition of the subject water. In no instance should the concentration of total dissolved materials exceed 50 milliosmoles (the equivalent of 1500 mg/l NaCl).

(2) Dissolved materials that are harmful in relatively low concentrations are discussed in the section "Toxicity."

pH, Alkalinity, Acidity

(1) No highly dissociated materials should be added in quantities sufficient to lower the pH below 6.0 or to raise the pH above 9.0.

(2) To protect the carbonate system and thus the productivity of the water, acid should not be added in sufficient quantity to lower the total alkalinity to less than 20 mg/l.

(3) The addition of weakly dissociated acids and alkalis should be regulated in terms of their own toxicities as established by bioassay procedures.

Temperature

Warm Water Biota: To maintain a well-rounded population of warm-water fishes, the following restrictions on temperature extremes and temperature increases are recommended:

(1) During any month of the year heat should not be added to a stream in excess of the amount that will raise the temperature of the water (at the expected minimum daily flow for that month) more than 5 F. In lakes, the temperature of the epilimnion in those areas where important organisms are most likely to be adversely affected should not be raised more than 3 F above that which existed before the addition of heat of artificial origin. The increase should be based on the monthly average of the maximum daily temperature. Unless a special study shows that a discharge

of a heated effluent into the hypolimnion will be desirable, such practice is not recommended and water for cooling should not be pumped from the hypolimnion to be discharged to the same body of water.

(2) The normal daily and seasonal temperature variations that were present before the addition of heat due to other than natural causes should be maintained.

(3) The recommended maximum temperatures that are not to be exceeded for various species of warm-water fish are given in table III-1.

Cold Water Biota: Because of the large number of trout and salmon waters which have been destroyed, made marginal, or nonproductive, remaining trout and salmon waters must be protected if this resource is to be preserved.

Inland trout streams, headwaters of salmon streams, trout and salmon lakes, and the hypolimnion of lakes and reservoirs containing salmonids and other cold water forms should not be warmed or used for cooling water. No heated effluents should be discharged in the vicinity of spawning areas.

For other types and reaches of cold-water streams, reservoirs and lakes, the following restrictions are recommended:

(1) During any month of the year heat should not be added to a stream in excess of the amount that will raise the temperature of the water more than 5 F (based on the minimum expected flow for that month). In lakes, the temperature of the epilimnion should not be raised more than 3 F by the addition of heat of artificial origin.

(2) The normal daily and seasonal temperature fluctuations that existed before the addition of heat due to other than natural causes should be maintained.

TABLE III-1

[Provisional maximum temperatures recommended as compatible with the well-being of various species of fish and their associated biota]

93 F:	Growth of catfish, gar, white or yellow bass, spotted bass, buffalo, carpsucker, threadfin shad, and gizzard shad.
90 F:	Growth of largemouth bass, drum, bluegill, and crappie.
84 F:	Growth of pike, perch, walleye, smallmouth bass, and sauger.
80 F:	Spawning and egg development of catfish, buffalo, threadfin shad, and gizzard shad.
75 F:	Spawning and egg development of largemouth bass, white and yellow bass, and spotted bass.
68 F:	Growth or migration routes of salmonids and for egg development of perch and smallmouth bass.
55 F:	Spawning and egg development of salmon and trout (other than lake trout).
48 F:	Spawning and egg development of lake trout, walleye, northern pike, sauger, and Atlantic salmon.

Note.—Recommended temperatures for other species, not listed above, may be established if and when necessary information becomes available.

(3) The recommended maximum temperatures that are not to be exceeded for various species of cold-water fish are given in table III-1.

Dissolved Oxygen

The following environmental conditions are considered essential for maintaining native populations of fish and other aquatic life.

(1) For a diversified warm-water biota, including game fish, DO concentration should be above 5 mg/l, assuming normal seasonal and daily variations are above this concentration. Under extreme conditions, however, they may range between 5 and 4 mg/l for short periods during any 24-hour period, provided that the water quality is favorable in all other respects. In stratified lakes, the DO requirements may not apply to the hypolimnion. In shallow unstratified lakes, they should apply to the entire circulation water mass.

These requirements should apply to all waters except administratively established mixing zones. In lakes, such zones must be restricted so as to limit the effect on the biota. In streams, there must be adequate and safe passageways for migrating forms. These must be extensive enough so that the majority of plankton and other drifting organisms are protected (see section on zones of passage).

(2) For the cold-water biota, it is desirable that DO concentrations be at or near saturation. This is especially important in spawning areas where DO levels must not be below 7 mg/l at any time. For good growth and the general well-being of trout, salmon, and their associated biota, DO concentrations should not be below 6 mg/l. Under extreme conditions, they may range between 6 and 5 mg/l for short periods provided the water quality is favorable in all other respects and normal daily and seasonal fluctuations occur. In large streams that have some stratification or that serve principally as migratory routes, DO levels may range between 4 and 5 mg/l for periods up to 6 hours, but should never be below 4 mg/l at any time or place.

(3) DO levels in the hypolimnion of oligotrophic small inland lakes and in large lakes should not be lowered below 6 mg/l at any time due to the addition of oxygen-demanding waste or other materials.

Carbon Dioxide

According to our present knowledge of the subject, it is recommended that the "free" carbon dioxide concentration should not exceed 25 mg/l.

Oil

Oil or petrochemicals should not be added in such quantities to the receiving waters that they will—

of a heated effluent into the hypolimnion will be desirable, such practice is not recommended and water for cooling should not be pumped from the hypolimnion to be discharged to the same body of water.

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(1) During any month of the year heat should not be added to a stream in excess of the amount that will raise the temperature of the water more than 5 F (based on the minimum expected flow for that month). In lakes, the temperature of the epilimnion should not be raised more than 3 F by the addition of heat of artificial origin.

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The following environmental conditions are considered essential for maintaining native populations of fish and other aquatic life.

(1) For a diversified warm-water biota, including game fish, DO concentration should be above 5 mg/l, assuming normal seasonal and daily variations are above this concentration. Under extreme conditions, however, they may range between 5 and 4 mg/l for short periods during any 24-hour period, provided that the water quality is favorable in all other respects. In stratified lakes, the DO requirements may not apply to the hypolimnion. In shallow unstratified lakes, they should apply to the entire circulation water mass.

These requirements should apply to all waters except administratively established mixing zones. In lakes, such zones must be restricted so as to limit the effect on the biota. In streams, there must be adequate and safe passageways for migrating forms. These must be extensive enough so that the majority of plankton and other drifting organisms are protected (see section on zones of passage).

(2) For the cold-water biota, it is desirable that DO concentrations be at or near saturation. This is especially important in spawning areas where DO levels must not be below 7 mg/l at any time. For good growth and the general well-being of trout, salmon, and their associated biota, DO concentrations should not be below 6 mg/l. Under extreme conditions, they may range between 6 and 5 mg/l for short periods provided the water quality is favorable in all other respects and normal daily and seasonal fluctuations occur. In large streams that have some stratification or that serve principally as migratory routes, DO levels may range between 4 and 5 mg/l for periods up to 6 hours, but should never be below 4 mg/l at any time or place.

(3) DO levels in the hypolimnion of oligotrophic small inland lakes and in large lakes should not be lowered below 6 mg/l at any time due to the addition of oxygen-demanding waste or other materials.

Carbon Dioxide

According to our present knowledge of the subject, it is recommended that the "free" carbon dioxide concentration should not exceed 25 mg/l.

Oil

Oil or petrochemicals should not be added in such quantities to the receiving waters that they will—

cation factor applied to determine the permissible concentration of toxicant

(2) **Pesticides.**

(a) **Chlorinated hydrocarbons:** Any addition of chlorinated hydrocarbon insecticides is likely to cause damage to some desired organisms and should be avoided.

(b) **Other chemical pesticides:** Addition of other kinds of chemicals used as pesticides and herbicides can cause damage to desirable organisms and should be applied with utmost discretion and caution. Table III-5 (p. 62) lists the 48-hour TL_m values of a number of pesticides for various types of fresh water organisms. To provide reasonably safe concentrations of these materials in receiving waters, application factors ranging from $\frac{1}{10}$ to $\frac{1}{100}$ should be used with these values depending on the characteristic of the pesticide in question and used as specified in (4), below. Concentrations thus derived may be considered tentatively safe under the conditions specified.

(3) **Other Toxic Substances.**

(a) **ABS:** Concentration of continuous exposure to ABS should not exceed $\frac{1}{4}$ of the 48-hour TL_m . A concentration as high as 1 mg/l may be tolerated occasionally for periods of time not exceeding 24 hours. ABS may increase the toxicity of other materials.

(b) **LAS:** The concentration of LAS should not exceed 0.2 mg/l or $\frac{1}{4}$ of the 48-hour TL_m .

(4) **Application Factors:** Concentration of materials that are nonpersistent (that is, have a half-life of less than 96 hours) or have noncumulative effects after mixing with the receiving waters should not exceed $\frac{1}{10}$ of the 96-hour TL_m value at any time or place. The 24-hour average of the concentration of these materials should not exceed $\frac{1}{20}$ of the TL_m value after mixing. For other toxicants the concentrations should not exceed $\frac{1}{20}$ and $\frac{1}{100}$ of the TL_m value under the conditions described above. Where specific application factors have been determined, they will be used in all instances.

(5) **General Considerations.** When two or more toxic materials that have additive effects are present at the same time in the receiving water, some reduction is necessary in the permissible concentrations as derived from bioassays on individual substances or wastes. The amount of reduction required is a function of both the number of toxic materials present and their concentrations in respect to the derived permissible concentration. An appropriate means of assuring that the combined amounts of the several substances do not exceed a permissible concentration for the mixture is through the use of following relationship:

$$\left(\frac{C_a}{L_a} + \frac{C_b}{L_b} + \dots + \frac{C_n}{L_n} \leq 1 \right)$$

Where $C_a, C_b, \dots, C_n$ are the measured concentrations of the several toxic materials in the water and $L_a, L_b, \dots, L_n$ are the respective permissible concentration limits derived for the materials on an individual basis. Should the sum of the several fractions exceed one, then a local restriction on the concentration of one or more of the substances is necessary.

Marine and estuarine organisms

Salinity

To protect estuarine organisms, no changes in channels, basin geometry, or freshwater influx should be made which would cause permanent changes in isohaline patterns of more than 10 percent of the naturally occurring variation.

Currents

Currents are important for transporting nutrients, larvae, and sedimentary materials for flushing and purifying wastes, and for maintaining patterns of scour and fill. To protect these functions, there should be no changes in basin geometry or freshwater inflow that will alter current patterns in such a way as to adversely affect existing biological and sedimentological situations.

pH

No materials that extend normal ranges of pH at any location by more than 0.1 pH unit should be introduced into salt water portions of tidal tributaries or coastal waters. At no time should the introduction of foreign materials cause the pH to be less than 6.7 nor greater than 8.5.

Temperature

In view of the requirements for the well-being and production of marine organisms, it is concluded that the discharge of any heated waste into any coastal or estuarine waters should be closely managed. Monthly means of the maximum daily temperatures recorded at the site in question and before the addition of any heat of artificial origin should not be raised by more than 4 F during the fall, winter, and spring (September through May), or by more than 1.5 F during the summer (June through August), North of Long Island and in the waters of the Pacific Northwest (north of California), summer limits apply July through September; and fall, winter, and spring limits ap-

ply October through June. The rate of temperature change should not exceed 1 F per hour except when due to natural phenomena.

Suggested temperatures are to prevail outside of established mixing zones as discussed in the section on zones of passage.

Dissolved Oxygen

Oxygen levels sufficient for the survival, growth, reproduction, general well-being, and production of a suitable crop must be maintained. The dissolved oxygen concentrations necessary to attain this objective in coastal waters, estuaries, and tidal tributaries are:

(1) Dissolved oxygen concentrations in surface coastal waters should be greater than 5.0 mg/l except when upwellings and other natural phenomena may cause this value to be depressed.

(2) Dissolved oxygen concentration in estuaries and tidal tributaries should not be less than 4.0 mg/l at any time or place except in naturally dystrophic waters or where natural conditions cause DO to be depressed.

Oil

No oil or petroleum products should be discharged into estuarine or coastal waters in quantities that: (1) Can be detected as a visible film, sheen, or by odor; (2) cause tainting of fish or edible invertebrates; (3) form an oil sludge deposit on the shores or bottom of the receiving body of water; (4) become effective toxicants according to the criteria recommended in the "Toxicity" section.

Turbidity

No effluent that may cause changes in turbidity or color should be allowed to enter estuarine or coastal waters unless it can be shown to have no deleterious effects on the aquatic biota.

Settleable and Floating Substances

No materials that contain settleable solids or substances that may precipitate out in quantities that adversely affect the biota should be introduced into coastal or estuarine waters. It is especially urgent that areas which serve as habitat or nursery grounds for commercially important species be protected from any impairment of natural conditions.

Tainting Substances

Substances that taint or produce off-flavors in fish and edible invertebrates should not be present in concentrations discernible by bioassay or organoleptic tests.

Radionuclides

The recommendations made for freshwater organisms apply to marine and estuarine organisms.

Plant Nutrients and Nuisance Organisms

(1) No changes should be made in the basin geometry, current structure, salinity, or temperature of the estuary until studies have shown that these changes will not adversely affect the biota or promote the increase of nuisance organisms.

(2) The artificial enrichment of the marine environment from all sources should not cause any major quantitative or qualitative alteration in the flora such as the production of persistent blooms of phytoplankton (whether toxic or not), dense growths of attached algae or higher aquatics, or any other sort of nuisance that can be attributed directly to nutrient excess or imbalance. Because these nutrients often are derived largely from drainage from land, special attention should be given to correct land management in river basins and shores of embayments to control unavoidable erosion.

(3) The naturally occurring atomic ratio of $\text{NO}_3\text{-N}$ to $\text{PO}_4\text{-P}$ in a body of water should be maintained. Similarly, the ratio of inorganic phosphorus (orthophosphate) to total phosphorus (the sum of inorganic phosphorus, dissolved organic phosphorus, and particulate phosphorus) should be maintained as it occurs naturally. Nutrient imbalances have been shown to cause a change in the natural diversity of desirable organisms and to reduce productivity.

Toxic Substances

(1) **Substances of Unknown Toxicity:** All effluents containing foreign materials should be considered harmful and not permissible until bioassay tests have shown otherwise. It should be the obligation of the agency producing the effluent to demonstrate that it is harmless in the concentrations that will be found in the receiving waters. All bioassays should be conducted strictly as recommended in the body of this report and the appropriate application factor applied to determine the permissible concentration of toxicant.

(2) **Pesticides for Which Limits Have Been Determined:** The pesticides are grouped according to their relative toxicity to shrimp. Criteria are based on the best estimates in the light of present knowledge and it is to be expected that acceptable levels of toxic materials may be changed as a result of future research.

Pesticide group A.—The following chemicals are acutely toxic at concentrations of 5 µg/l and less. On the assumption that $\frac{1}{100}$ of this level represents a reasonable application factor, it is recommended that environmental levels of these substances not be permitted to rise above 50 nanograms/l. This level is so low that these pesticides could not be applied directly in or near the marine habitat without danger of causing damage. The 48-hour TL_m is listed for each chemical in µg/l.

Organochloride pesticides

Aldrin	0.04	DDT	0.6
BHC	2.0	Dieldrin	0.3
Chlordane	2.0	Endosulfan	0.2
Endrin	0.2	Methoxychlor	4.0
Heptachlor	0.2	Perthane	3.0
Lindane	0.2	TDE	3.0
		Toxaphene	3.0

Organophosphorus pesticides

Coumaphos	2.0	Naled	3.0
Dursban	3.0	Parathion	1.0
Fenthion	0.03	Ronnel	5.0

Pesticide group B.—The following types of pesticide compounds are generally not acutely toxic at levels of 1.0 mg/l or less. It is recommended that an application factor of $\frac{1}{100}$ be used and in the absence of acute toxicity data that an environmental level of not more than 10 µg/l be permitted. An acute toxicity factor must be established for each specific chemical in this group to determine that it is not more toxic than related compounds as indicated above:

Arsenicals	2,4,5-T compounds.
Botanicals	Phthalic acid compounds.
Carbamates	Triazine compounds.
2,4-D compounds	Substituted urea compounds.

Other Pesticides.—Acute toxicity data are available for approximately 100 technical-grade pesticides in general use not listed in the above groups. These chemicals are either not likely to reach the marine environment or, if used as directed by the registered label, probably would not occur at levels toxic to marine biota. It is presumed that criteria established for these chemicals in fresh water will protect adequately the marine habitat. It should be emphasized that no unlisted chemical should be discharged into the estuary without preliminary bioassay tests.

(3) Industrial and Other Toxic Wastes.

(a) Safe concentrations of metals, ammonia, cyanide, and sulfide should be determined by the use of appropriate application factors to 96-hour TL_m values as determined by flow-through bioassays using dilution water that came from the re-

ceiving body. Test organisms should be local species or life stages of organisms of economic and ecologic importance which are the most sensitive to the waste in question. Application factors should be $\frac{1}{100}$ for metals, $\frac{1}{20}$ for ammonia, $\frac{1}{10}$ for cyanide, and $\frac{1}{20}$ for sulfide.

(b) Fluoride concentrations should not exceed those for drinking water.

(c) Permissible levels of detergents in fresh waters should also be applied to the marine and estuarine waters.

(d) Bacteriological criteria of estuarine waters utilized for shellfish cultivation and harvesting should conform with the standards as described in the National Shellfish Sanitation Program Manual of Operation. These standards provide that—

(1) examinations shall be conducted in accordance with the American Public Health Association recommended procedures for the examination of sea water and shellfish;

(2) there shall be no direct discharges of untreated sewage;

(3) samples of water for bacteriological examination to be collected under those conditions of time and tide which produce maximum concentration of bacteria;

(4) the coliform median MPN of the water does not exceed 70/100 ml, and not more than 10 percent of the samples ordinarily exceed an MPN of 230/100 ml for a five-tube decimal dilution test (or 330/100 ml, where the three-tube decimal dilution test is used) in those portions of the area most probably exposed to fecal contamination during the most unfavorable hydrographic and pollution conditions;

(5) the reliability of nearby waste treatment plants shall be considered in the approval of areas for direct harvesting.

(e) Wastes from tar, gas, coke, petrochemical, pulp and paper manufacturing, waterfront and boating activities, hospitals, marine laboratories and research installation wastes are all complex mixtures having great variability in character and toxicity. Due to this variability, safe levels must be determined at frequent intervals by flow-through bioassays of the individual effluents.

For those operations having persistent toxicants, an application factor of $\frac{1}{100}$ should be used while for those composed largely of unstable or biodegradable toxicants, an application factor of $\frac{1}{20}$ is tentatively suggested.

(4) General Considerations.—When two or more toxicants that have additive effects are present, they must be treated as suggested earlier under fresh water organisms.

Wildlife

Dissolved Oxygen

In addition to the DO requirements for aquatic organisms, the bottoms of areas used by wildfowl must be kept aerobic to suppress botulinus organisms.

pH

Aquatic plants of greatest value as food for waterfowl thrive best in waters with a summer pH range of 7.0 to 9.2.

Alkalinity

Waterfowl habitats, to be productive, should have a bicarbonate alkalinity between 30 and 130 mg/l. Fluctuations should be less than 50 mg/l from natural conditions.

Salinity

Salinity should be kept as close to natural conditions as possible. Fluctuations in salinity during any 24-hour period should be limited as follows:

Natural salinity:	Variation permitted
0 to 3.5‰	1‰
3.5 to 13.5‰	2‰
13.5 to 35.0‰	4‰

Light Penetration

Optimum light requirements for aquatic wildlife habitats should be at least 10 percent of incident light at the surface to a 6-foot depth; the tolerable limit should be 5 percent of the light at the surface to the same depth.

Settleable Substances

Settleable substances destroy the usefulness of aquatic bottoms for waterfowl. Settleable substances should be excluded from areas expected to support waterfowl.

Oil

Oil is an especially dangerous substance to waterfowl. Oil and petrochemicals must be excluded from both the surface and bottoms of any area used by waterfowl.

Toxic Substances

Toxic substances should be excluded from wildlife habitats to the degree that they affect the health and well-being of wildlife, either directly or

through biological magnification. Special consideration must be given to keep edible wildlife safe for consumption by humans.

Disease

Offal from poultry houses, meatpacking plants, as well as other possible sources of disease organisms, must be excluded from areas supporting wildlife to guard against transmission of such diseases as botulism, fowl cholera, and aspergillosis.

General

Water quality suitable for fish and other aquatic organisms will be adequate for wildlife.

The preceding is an abridgement. For the complete statement of the Office of Water Programs Criteria for Fish, Other Aquatic Life, and Wildlife, see "Water Quality Criteria, Report of the National Technical Advisory Committee," April 1, 1968.

WATER QUALITY CRITERIA

AGRICULTURAL USES

Summary and Key Criteria

FARMSTEAD WATER SUPPLIES

In view of the wide variety of sources used for farmstead water supplies and uncontrolled influences of geographic location and climatic conditions, no single set of values can realistically be established as criteria for farmstead supplies and accordingly most of the values are given in the form of acceptable ranges at point of use. In developing the criteria summarized in table IV-1, considerable reliance has been placed on the U.S. Public Health Service Drinking Water Standards. Water which meets these standards is generally safe and acceptable to the user. Farmstead water supply includes water to be used for all household purposes, washing of raw agricultural commodities, and for milk sanitation. Specific requirements above those for general farmstead use are indicated also. This summary should be used only in conjunction with the test of this report and appropriate references. See table IV-1, below.

LIVESTOCK WATER SUPPLIES

Available literature on various substances which occur as contaminants of drinking water for livestock has been reviewed. Such contaminants include inorganic elements and their salts, organic wastes and mill effluents, pathogens and parasitic organisms, herbicide and pesticide residues, and radionuclides. Important and significant variables, including nature and intake of dietary dry matter, species, age and productivity of animals, and interrelationships among the contaminating ingredients, make establishment of a single set of water purity values for livestock unfeasible. The relationship of water intake to total dietary intake by livestock is of particular significance. For example, much lower levels of a toxic contaminant should be set for water if the dry feed to be ingested is unavoidably high in the same substance. In general, the risk of toxicity is less from water than from feed sources.

Employment of biological indicators, such as fish in livestock water supplies is proposed as a means of monitoring their safety from the standpoint of chemical toxicity. Fish do not, however, normally indicate presence of pathogenic organisms with sufficient sensitivity to protect livestock from these contaminants.

Animal pathogens may occasionally enter into a water cycle and management of water resources can materially influence distribution of some diseases. The involvement of the water supply in such cases should be supported by epidemiological studies in addition to presumptive or definitive isolation from the water environment. Danger from certain microbiological pathogens may be increased in situations where water supplies are alkaline.

In some instances, water quality standards are set in conformity with accepted residual levels in marketable animal tissues or products rather than in relation to any demonstrable toxic effect upon the animal themselves.

IRRIGATION WATER SUPPLIES

Variations and interactions of soils, plants, water, and climate preclude the establishment of a single set of criteria to evaluate all water quality characteristics for irrigation purposes. It is the intent of this report to list tentative criteria where possible and to suggest guidelines where specific criteria cannot be defined. These recommendations are subject to revision as more knowledge accumulates.

The following summary of criteria should be used in conjunction with references to the text of the report as noted.

Salinity

Arid and Semiarid Regions

Table IV-3 of recommended criteria for salinity or total dissolved solids (TDS) assumes that related factors set forth in this report are taken into consideration. This includes good irrigation practices and soil and plant variables (pp. 167-171).

Humid Regions

Where irrigation is practical in humid areas, it is unlikely that any great accumulations of salt will occur over a period of time. For this reason, criteria suggested above for arid regions may be significantly increased for humid regions. More appropriate criteria are indicated in pages 173-174.

TABLE IV-1. Key Water Quality Criteria for Farmstead Uses.

Characteristic	Recommendations (at point of use)		Page
	General farmstead uses	Additional special-use requirements	
Taste and odor	Substantially free		124
Color	do		124
pH	6.0 to 8.5	6.8 to 8.5 dairy sanitation	124
Total dissolved inorganic solids	500 mg/l (under certain circumstances, higher levels are acceptable)		124
Dissolved organic compounds	No recommendations for total organics. The concentration of persistent chlorinated organic pesticides should not exceed the following: Compound $\mu\text{g/l}$ Endrin 1 Aldrin 17 Dieldrin 17 Lindane 56 Toxaphene 5 Heptachlor 18 H. epoxide 42 DDT 3 Chlordane 35 Methoxychlor 35		124
Turbidity	Substantially free		125
Hazardous trace elements	Levels in excess of those shown are grounds for rejection of a supply: Substances mg/l Arsenic 0.05 Barium 1.00 Cadmium 0.01 Chromium 0.05 Cyanides 0.2 Lead 0.05 Selenium 0.01 Silver 0.05		125
Other trace elements	Levels shown below should not be exceeded if alternate sources are available: Substances mg/l Manganese 0.05 Iron 0.3 Copper 1.0 Zinc 5.0 Fluoride 0.7-1.2 Nitrate 45.0 pc/l Strontium 90 10 Radium 226 3 In absence of above radionuclides, 1,000 pc/l gross β activity.	In dairy sanitation, water should contain < 20 mg/l potassium and < 0.1 mg/l iron and copper.	125
Radionuclides			125
Nonpathogenic microorganisms	To conform to USPHS drinking water standards.	For dairy sanitation, water should not contain more than 20 organisms per ml and contain not more than 5 typhoid and/or proteolytic organisms per ml.	125

SAR and Sodium

Water having sodium adsorption ratio (SAR) values between 8 and 18 may have an adverse effect on the permeability of soils containing an appreciable proportion of clay because its use causes undesirable amounts of sodium to be adsorbed. Where used on sensitive crops, SAR values above 4 may be detrimental because of sodium phytotoxicity (pp. 155, 164). Water low in salt but high in bicarbonate content may present a permeability hazard even with low SAR values (pp. 170-171).

TABLE IV-2. Key Water Quality Criteria for Livestock Use

Characteristic	Recommendations
Total dissolved solids (TDS)	<10,000 mg/l, depending upon animal species and ionic composition of the water.
Hazardous trace elements:	
Arsenic	<0.05 mg/l
Cadmium	<0.01 mg/l
Chromium	<0.05 mg/l
Fluorine	<2.40 mg/l
Lead	<0.05 mg/l
Selenium	<0.01 mg/l
Organic substances:	
Algae (water bloom)	Avoid abnormally heavy growth of blue-green algae.
Parasites and pathogens.	Conform to epidemiological evidence.
Dissolved organic compounds.	Biological accumulation from environmental sources, including water, shall not exceed established, legal limits in livestock products.
Radionuclides	Conform to recommendations for farmstead water supplies.

TABLE IV-3. Suggested Guidelines for Salinity in Irrigation Water

Crop response	TDS mg/l	EC mmhos/cm
Water for which no detrimental effects will usually be noticed	500	<0.75
Water which can have detrimental effects on sensitive crops	500 1,000	0.75 1.50
Water that may have adverse effects on many crops and requiring careful management practices	1,000 2,000	1.50 3.00
Water that can be used for salt tolerant plants on permeable soils with careful management practices	2,000 5,000	3.00 7.50

Electrical conductivity.

Chlorides

Although not phytotoxic to most crops, some chloride phytotoxicity has been found for some fruit crops. No limit has been established for chloride-tolerant crops because detrimental effects from salinity *per se* ordinarily deter crop growth first. For chloride-sensitive crops, chloride content in the soil solution may range from 10 to 50 me/l with permissible levels in irrigation water ranging from 1 to 20 me/l (16). More restrictive criteria should be considered where sprinkler irrigation is used (pp. 155-156).

Trace Elements

Toxic limits which would be generally applicable to all soils and all crops are not easily defined. Research literature is inadequate to permit even well-defined guidelines. The limits suggested in table IV-15 are tentative and are designed only to serve as guides for well-drained soils (p. 152).

Radionuclides

There are many considerations involved regarding radioactivity in irrigation water (pp. 163-164). One hazard is the potential accumulation of a radionuclide in a soil reaching levels in excess of that applied in the irrigation water. On the basis of existing knowledge, USPHS Drinking Water Standards (175) is the best guide; the standards are: Strontium-90, 10 pc/l; radium-226, 3 pc/l. In the absence of these radionuclides, 1,000 pc/l gross beta activity.

Microorganisms

It is impractical to monitor irrigation water for the numerous pathogenic organisms which may be present (pp. 160-163). For this reason, the following guidelines for coliform limitations are suggested for interim use subject to research confirmation. These are especially applicable where the tops or roots of the irrigated crop are to be consumed directly by man or livestock.

The monthly arithmetic average density of the coliform group of bacteria shall not exceed 5,000 per 100 milliliters and the monthly arithmetic average density of fecal coliforms shall not exceed 1,000 per 100 milliliters. Both of these limits shall be an average of at least two consecutive samples examined per month during the irrigation season and any one sample examined in any 1 month shall not exceed a coliform group density of more than 20,000 per 100 milliliters, or a fecal coliform density of more than 4,000 per 100 milliliters.

For the control of plant pathogens, guidelines for irrigation water are best framed in terms of preventive measures rather than by assay procedures.

pH

Acidity or alkalinity as such in irrigation water is seldom directly detrimental to crop growth. Normally, water with pH values of 4.5 to 9.0 should not present any insurmountable problems, but a range of 5.5 to 8.5 would be more desirable (p. 155).

Temperature

Excessively high or low temperatures in irrigation water may affect crop growth and yields (pp. 157, 160). A desirable range of water temperatures is from 55 to 85 F.

Suspended Solids

Sediment and suspended solids may be detrimental in irrigation water because of their effect on irrigation structures and equipment and on the soil to which the water is applied. No guidelines are available to establish standards for either particle size or quantity. (pp. 163, 175).

Pesticides

On the basis of the limited information available, levels of herbicides at which crop injury has been observed are shown in Table IV-4. There is little evidence to indicate that other pesticide contamination of irrigation water would be detrimental to plant growth or accumulate in or on edible plants in toxic concentrations under normal use (pp. 156-157).

Biochemical Oxygen Demand (BOD) and Dissolved Oxygen

Insufficient information is available to suggest guidelines or to indicate that low BOD values or dissolved oxygen content of an irrigation water as such will have a deleterious effect on plant growth or well-drained soils.

TABLE IV-4
Levels of Herbicides in Irrigation Water at Which Crop Injury Has Been Observed¹

Herbicide	Crop Injury Threshold in Irrigation Water mg/l
Acrolein	Flood or furrow: beans-60, corn-60, cotton-80, soybeans-20, sugar beets-60. Sprinkler: corn-60, soybeans-15, sugar beets-15.
Aromatic solvents (xylene)	Alfalfa-> 1,600, beans-1,200, carrots-1,600, corn-3,000, cotton- 1,600, grain sorghum-> 800, oats-2,400, potatoes-1,300, wheat-1,200.
Copper sulfate	Apparently, above concentrations used for weed control.
Amitrole-T	Beets (rutabaga)->3.5, corn->3.5.
Dalapon	Beets->7.0, corn-<0.35.
Diquat	Beans-5.0, corn-125.0.
Endothall Na and K salts	Corn-25, field beans-<1.0, alfalfa->10.0.
Dimethylamines	Corn->25, soybeans->25, sugar beets-25.
2, 4-D	Field beans->3.5 <10, grapes-0.7-1.5, sugar beets-3.5.
Dichlobenil	Alfalfa-10, corn->10, soybeans-1.0, sugar beets-1.0-10.
Fenac	Alfalfa-1.0, corn-10, soybeans-0.1, sugar beets-0.1-10.
Picloram	Corn->10, field beans-0.1, sugar beets-<1.0.

¹Data submitted by crops research division, ARS, USDA (unpublished)

NOTE: Where the symbol ">" is used, the concentration in water cause no injury.
Data are for furrow irrigation unless otherwise specified.

The preceding is an abridgement. For the complete statement of the Water Programs Operations Criteria for Agricultural Uses, See "Water Quality Criteria, Report of the National Technical Advisory Committee," April 1, 1968.

WATER QUALITY CRITERIA

INDUSTRY

I INTRODUCTION

A Scope

Water Quality requirements differ so widely for the hundreds of uses to which water is put industrially that no meaningful criteria for surface water supplies can encompass a majority of such uses. Furthermore, water treatment technology in its present state of development permits the utilization of surface water of literally any available quality to create waters of any desired quality at point of use. Such treatment may be costly, but this cost is usually a small part of the total production and marketing costs. The National Technical Advisory Subcommittee for Water Quality Requirements for Industrial Water Supplies has identified the appropriate quality characteristics of raw waters that have been used and the quality requirements of waters at the point of use for each industry.

Each value given in this report for a quality characteristic of raw water supplies for industrial purposes has occurred in water that has been used somewhere in this country. However, the characteristic values may be altered by treatment to produce the quality of water required at point of use. Hence, tables listing desired water quality criteria at the point of use prior to internal conditioning have been developed for each major industrial requirement.

B Industries Considered

The Subcommittee was subdivided into six task forces. Task force I was concerned with water used for cooling and steam generation for all industries. Each of the other task forces was assigned one or more industrial groups as defined by the 2-digit Standard Industrial Classification (SIC) coding used by the Bureau of Census (6).

Although it has not been feasible to cover all industries, the major users of water, including some industries, that require process waters having a wide range of quality, have been considered.

C Water Use

The total water intake of both industrial manufacturing plants and investor-owned thermal electric utilities was approximately 49,000 billion gallons during 1964. About 90 percent or 44,000 billion gallons per year (bgy) of all intake water was used for cooling or condensing purposes. Water used for processing, including water coming into contact with the product as steam or as coolant, amounted to nearly 8 percent (3,700 bgy) of the total water intake. The remaining 2 percent (960 bgy) was used for boiler-feed water.

Brackish water, water containing more than 1,000 mg/l dissolved solids, amounted to nearly 30 percent (15,000 bgy) of the total-intake water. Most (32,000 bgy) of the fresh water intake (34,000 bgy) was surface water delivered by company-owned water systems.

The manufacturing industry used approximately 4,300 billion gallons of water that they treated or secured from a public supply in 1964. This was 30 percent of water intake for manufacturing and approximates 90 percent of all water that they used for boiler feed and processing.

D Raw Water Quality

In general, the procedure used by the individual task forces involved first establishing the quality requirements for various waters at point of use exclusive only of the addition of internal conditioning chemicals such as biocides,

Water Quality Criteria

or corrosion or deposit inhibitors. Second, the consideration of methods of external treatment (e.g. clarification, softening, demineralization, etc.) that have been used; and finally establishing the quality characteristics of raw surface waters that have been used by the various industries.

E Minimum Standards

Minimum standards that should be met by all surface waters for all uses include the following (3). The water should be:

- 1 Free from substances attributable to municipal, industrial, or other discharges, or agricultural practices that will settle to form putrescent or otherwise objectionable sludge deposits;

- 2 Free from floating debris, oil, scum, and other floating materials attributable to municipal, industrial, or other discharges, or agricultural practices in amounts sufficient to be unsightly or deleterious;

- 3 Free from materials attributable to municipal, industrial, or other discharges, or agricultural practices producing color, odor or other conditions in such a degree as to cause a nuisance.

Additional quality characteristics of surface waters that have been used as sources for industrial water supplies are summarized in Table V-3. The specific water characteristics are maximums and no water will have all of the maximum values shown.

TABLE V-3. Summary of Specific Quality Characteristics of Surface Waters That Have Been Used as Sources for Industrial Water Supplies

[Unless otherwise indicated, units are mg/l and values are maximums. No one water will have all the maximum values shown.]

Characteristic	Boiler makeup water		Cooling water				Process water								
	Industrial 0 to 1,500 psig	Utility 700 to 5,000 psig	Fresh		Brackish ¹		Textile industry, SIC-22	Lumber industry, SIC-24	Pulp and paper industry, SIC-26	Chemical industry, SIC-28	Petroleum industry, SIC-29	Prim. metals industry, SIC-33	Food and kindred products, SIC-20	Leather industry, SIC-31	
			Once through	Makeup recycle	Once through	makeup recycle									
Silica (SiO ₂)	150	150	50	150	25	25	-----	-----	50	-----	50	-----	For the above 2 categories the quality of raw surface supply should be that prescribed by the NTA Subcommittee on Water Quality Requirements for Public Water Supplies.		
Aluminum (Al)	3	3	3	3	-----	-----	-----	-----	-----	2.6	5	15			
Iron (Fe)	80	80	14	80	1.0	1.0	0.3	-----	-----	-----	-----	-----			
Manganese (Mn)	10	10	2.5	10	0.02	0.02	1.0	-----	-----	2	-----	-----			
Copper (Cu)	-----	-----	-----	-----	-----	-----	0.5	-----	-----	-----	-----	-----			
Calcium (Ca)	-----	-----	500	500	1,200	1,200	-----	-----	-----	200	220	-----			
Magnesium (Mg)	-----	-----	-----	-----	-----	-----	-----	-----	-----	100	85	-----			
Sodium and potassium (Na+K)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	230	-----			
Ammonia (NH ₃)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----			
Bicarbonate (HCO ₃)	600	600	600	600	180	180	-----	-----	-----	600	480	-----			
Sulfate (SO ₄)	1,400	1,400	680	680	2,700	2,700	-----	-----	-----	850	570	-----			
Chloride (Cl)	19,000	19,000	600	500	22,000	22,000	-----	-----	200 ²	500	1,600	500			
Fluoride (F)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	1.2	-----			
Nitrate (NO ₃)	-----	-----	30	30	-----	-----	-----	-----	-----	-----	8	-----			
Phosphate (PO ₄)	-----	50	4	4	5	5	-----	-----	-----	-----	-----	-----			
Dissolved solids	35,000	35,000	1,000	1,000	35,000	35,000	150	-----	1,080	2,500	3,500	1,500			
Suspended solids	15,000	15,000	5,000	15,000	250	250	1,000	(³)	-----	10,000	5,000	3,000			
Hardness (CaCO ₃)	5,000	5,000	850	850	7,000	7,000	120	-----	475	1,000	900	1,000			
Alkalinity (CaCO ₃)	500	500	500	500	150	150	-----	-----	-----	500	-----	200			
Acidity (CaCO ₃)	1,000	1,000	0	200	0	0	-----	-----	-----	-----	-----	75			
pH, units	-----	-----	5.0-8.9	3.5-9.1	5.0-8.4	5.0-8.4	6.0-8.0	5-9	4.6-9.4	5.5-9.0	6.0-9.0	3-9			
Color, units	1,200	1,200	-----	1,200	-----	-----	-----	-----	360	500	25	-----			
Organics:	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----			
Methylene blue active substances	2 ⁴	10	1.3	1.3	-----	1.3	-----	-----	-----	-----	-----	-----			
Carbon tetrachloride extract	100	100	(⁵)	100	(⁵)	100	-----	-----	-----	-----	-----	30			
Chemical oxygen demand (O ₂)	100	500	-----	100	-----	200	-----	-----	-----	-----	-----	-----			
Hydrogen sulfide (H ₂ S)	-----	-----	-----	-----	4	4	-----	-----	-----	-----	-----	-----			
Temperature, F	120	120	100	120	100	120	-----	-----	95 ⁶	-----	-----	100			

¹ Water containing in excess of 1,000 mg/l dissolved solids.

² May be $\leq$ 1,000 for mechanical pulping operations.

³ No large particles $\leq$ 3 mm diameter.

⁴ 1 mg/l for pressures up to 700 psig.

⁵ No floating oil.

⁶ Applies to bleached chemical pulp and paper only.

NOTE.—Application of the above values should be based on Part 23, ASTM book of standards (1) or APHA Standard methods for the examination of water and waste water (5).

Because of the very extensive use of water for cooling and boiler-feed purposes, the quality characteristics for surface waters for these purposes have been given special emphasis. In general, the surface water quality characteristics for process waters are applicable for the 2-digit SIC group of industries.

F Conclusions

The Subcommittee has reached the following conclusions regarding the water quality characteristics and requirements for industrial supplies.

- 1 The quality characteristics of the water supply for an established industry at a given site, if allowed to deteriorate from the range usually experienced for those characteristics of significance to that industry, can cause an undesirable increase in the cost for treatment. On the other hand, an improvement in the quality of the same supply will not significantly decrease the cost of treatment at an existing installation.
- 2 Marked variations in the quality of an industrial water supply can result in deterioration of product quality for some industries.
- 3 The water quality requirements at the point of use in each process in each industry as distinguished from the quality characteristics at the point of supply are generally well established for each existing industrial process use. These water quality requirements, however, vary considerably even for the same process depending upon the technological age of the design and other factors.
- 4 The quantity of water employed for process use by different plants in the same industry may vary considerably between plants depending on the cost of treatment, the age of the plant design, operating practices, and the quality and quantity of the available supply.

The preceding is an abridgement. For the complete statement of the Water Programs Operations Criteria for Industry, see "Water Quality Criteria, Report of the National Technical Advisory Committee," April 1, 1968.

II. DATA COLLECTION AND EVALUATION

	<u>Outline Number</u>
Statistics for Chemists	3
Accuracy-Precision-Error	4
Interlaboratory Quality Control Studies	5

STATISTICS FOR CHEMISTS

I INTRODUCTION

- A Statistics may be defined, for our purpose, as a collection of methods which have been developed for handling numerical data pertaining to samples or portions of entire populations.
- B The statistical methods with which we will concern ourselves deal with the presentation and analysis of numerical data from samples.

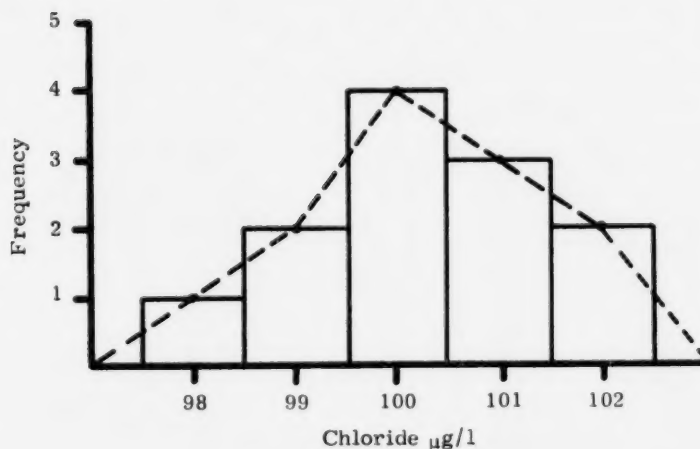
II FREQUENCY

A Definitions

- 1 Frequency - indicates how many times a particular score occurs in a collection of data
- 2 Frequency table - a tabular arrangement of data, ranked in ascending or descending order of magnitude, together with the corresponding frequencies
- 3 Frequency histogram - a set of rectangles having bases on a horizontal axis with centers at the given scores and heights equal to the corresponding frequencies (See Figure 1)
- 4 Frequency polygon - a line graph of frequencies plotted against scores (can be obtained by connecting mid-points of tops of rectangles in the frequency histogram) (See Figure 1)

Figure 1

Frequency Histogram & Frequency Polygon



B Application

Consider the application of the above definitions to the following set of data, obtained from twelve determinations for chloride in water.

Results ($\mu\text{g/l}$)		
100	101	99
101	100	100
99	102	100
98	101	102

Table 1

Frequency Table

Chloride ($\mu\text{g/l}$)	Frequency
98	1
99	2
100	4
101	3
102	2

III MEASURES OF CENTRAL TENDENCY

A Definitions

- 1 Central tendency - the tendency of values to cluster about a particular value in the distribution
- 2 Mode - that value which occurs most frequently
- 3 Median - midpoint of an array of scores. If there is an odd number of observations, n , the median is $\frac{X_{\frac{n+1}{2}}}{2}$ where $\frac{X_{\frac{n+1}{2}}}{2}$ represents the $\frac{n+1}{2}$ value in the frequency distribution. If there is an even

number of observations the median is $\frac{X_{\frac{n}{2}} + X_{\frac{n}{2} + 1}}{2}$, the average of the middle two scores.

- 4 Mean - arithmetic average of all the values in the sample distribution, denoted by $\bar{X}$. The formula for calculating the sample mean is

$$\bar{X} = \frac{X_1 + X_2 + X_3 + \dots + X_n}{n}$$

$$\bar{X} = \frac{\sum_{i=1}^n X_i}{n}$$

$$\bar{X} = \frac{\sum X_i}{n} \text{ where there are } n \text{ number of values.}$$

B Aids in calculation of the mean

Application of the following two statements can reduce errors and amount of time spent in calculating the mean of a distribution.

- 1 Adding or subtracting a constant to or from each score in a distribution is equivalent to adding or subtracting the same constant to or from the mean of the distribution. Thus the following formula:
- 2 Multiplying or dividing each score in a distribution by a constant is equivalent to multiplying or dividing the mean of the distribution by the same constant. Thus the following formulas:

$$\bar{X}_C = \bar{X} \pm C \text{ where the } X_i\text{'s are the values in the distribution with mean } \bar{X}, \text{ and the } X_i \pm C\text{'s are the values in the distribution with mean } \bar{X}_C.$$

$$(1) \bar{X}_C = C\bar{X}$$

or

$$(2) \bar{X}_C = \frac{\bar{X}}{C} \text{ where the } X_i\text{'s are the values in the distribution with mean } \bar{X},$$

and the CX_i 's or the $\frac{X_i}{C}$'s are the values in the distribution with mean $\bar{X}_c$

C Application

Consider the application of the above definitions to the previously mentioned set of data, obtained from twelve determinations for chloride in water, shown in Table 1.

1 Mode = 100

$$2 \text{ Median} = \frac{\frac{X_n}{2} + \frac{X_n}{2} + 1}{2} = \frac{X_6 + X_7}{2} \\ = \frac{100 + 100}{2} = 100$$

$$3 \text{ Mean} = \frac{\sum X_i}{n} \\ = \frac{98 + 2(99) + 4(100) + 3(101) + 2(102)}{12} \\ = 100.25$$

4 Aid in Calculation

Consulting Table 1 and observing that the values are in the neighborhood of 100 we might subtract 100 from each score and obtain the following distribution:

Table 2
Frequency Table

Chloride ($\mu\text{g/l}$)	Frequency
-2	1
-1	2
0	4
1	3
2	2

Denote the mean of the distribution in Table 1 by $\bar{X}_c$. If we add 100 to each score in the distribution in Table 2, we obtain the scores in the distribution in Table 1; likewise if we add 100 to the mean, $\bar{X}$, of the distribution in Table 2, we obtain the mean, $\bar{X}_c$, of the distribution in Table 1.

$$\text{Thus } \bar{X}_c = \bar{X} + 100$$

$$\bar{X}_c = \frac{\sum X}{n} + 100$$

$$\bar{X}_c = \frac{1(-2) + 2(-1) + 4(0) + 3(1) + 2(2)}{12} + 100$$

$$\bar{X}_c = .25 + 100 = 100.25$$

IV MEASURES OF DISPERSION

A Definitions

- 1 Dispersion - spread or variability of observations in a distribution
- 2 Range - the difference between the highest value and the lowest value
- 3 Average deviation - the sum of the deviations of the values from their mean, without regard to sign, divided by the total number of data values (n)

The formula for calculating the average deviation is:

$$d = \frac{\sum |X_i - \bar{X}|}{n}$$

- 4 Average deviation of the mean (D) - the average deviation of individual data items from the mean (d) divided by the square root of the number of data items (n)

The definition of the average deviation of the mean can be expressed by the formula:

$$D = \frac{d}{\sqrt{n}}$$

- 5 Variance - the sum of the squares of the deviations of the values from their mean divided by the total number of data values (n) minus 1

The definition of the variance can be expressed by the following formula:

$$s^2 = \frac{\sum (X_i - \bar{X})^2}{n - 1}$$

- 6 Standard deviation - the square root of the variance

The definition of the standard deviation can be expressed by the following formula:

$$s = \sqrt{\frac{\sum (X_i - \bar{X})^2}{n - 1}}$$

However, the formula commonly used because of its adaptability to the hand calculator is the following:

$$s = \sqrt{\frac{\sum X_i^2 - \frac{(\sum X_i)^2}{n}}{n - 1}} \quad \text{where there are } n \text{ number of values.}$$

- 7 Standard deviation of the mean (S) - the standard deviation of individual data items (s) divided by the square root of the number of data items (n)

The definition of the standard deviation of the mean can be expressed by the formula:

$$S = \frac{s}{\sqrt{n}}$$

- 8 Relative standard deviation - the standard deviation (s) expressed as a fraction of the mean, $\frac{s}{\bar{X}}$

The relative standard deviation is often expressed as a percent. It is then referred to as the coefficient of variation (V):

$$V = \frac{s}{\bar{X}} \times 100 = \%$$

The relative standard deviation is particularly helpful when comparing the precision of a number of determinations on a given substance at different levels of concentration.

B Aids in Calculation

Application of the following statements can reduce errors and amount of time spent in calculating the variance or standard deviation of a distribution.

- 1 Adding or subtracting a constant to or from each score in a distribution doesn't affect the variance or standard deviation of the distribution.

Thus the following formulas:

$$(1) \quad s_c^2 = s^2$$

$$(2) \quad s_c = s$$

where the X_i 's are the values in the distribution with variance s^2 and standard deviation s , and the $X_i + C$'s are the values in the distribution with variance s_c^2 and standard deviation s_c .

- 2 Multiplying or dividing each score in a distribution by a constant is equivalent to multiplying or dividing the variance of that distribution by the square of the same constant.

Thus the following formulas:

$$(1) s_c^2 = C^2 s^2$$

$$(2) s_c^2 = \frac{s^2}{C^2}$$

where the X_i 's are the values in the distribution with variance s^2 , and the CX_i 's or the $\frac{X_i}{C}$'s are the values in the distribution with variance s_c^2 .

- 3 Multiplying or dividing each score in a distribution by a constant is equivalent to multiplying or dividing the standard deviation of that distribution by the same constant.

Thus the following formulas:

$$(1) s_c = Cs$$

or

$$(2) s_c = \frac{s}{C}$$

where the X_i 's are the values in the distribution with standard deviation s , and the CX_i 's or the $\frac{X_i}{C}$'s are the values in the distribution with standard deviation s_c .

C Application

Consider the application of the above definitions to the previously mentioned set of data, obtained from twelve determinations for chloride in water, shown in II B, Table 1.

$$1 \text{ Range} = 102 - 98 = 4$$

$$2 \text{ Average deviation} - d = \frac{\sum |X_i - \bar{X}|}{n}$$

n	X_i	$ X_i - \bar{X} $	$n X_i - \bar{X} $
1	98	2.25	2.25
2	99	1.25	2.50
4	100	.25	1.00
3	101	.75	2.25
2	102	1.75	3.50
$\bar{X} = 100.25$			11.50

$$d = \frac{\sum |X_i - \bar{X}|}{n} = \frac{11.50}{12} = .96$$

- 3 Average deviation of the mean -

$$D = \frac{d}{\sqrt{n}}$$

Using calculations from number 2,

$$D = \frac{d}{\sqrt{n}} = \frac{0.96}{\sqrt{12}} = \frac{0.96}{3.46} = 0.28$$

$$4 \text{ Variance} - s^2 = \frac{\sum (X_i - \bar{X})^2}{n-1}$$

n	X_i	$X_i - \bar{X}$	$(X_i - \bar{X})^2$	$n(X_i - \bar{X})^2$
1	98	-2.25	5.06	5.06
2	99	-1.25	1.56	3.12
4	100	-.25	.06	.24
3	101	+.75	.56	1.68
2	102	+1.75	3.06	6.12
				16.22

$$s^2 = \frac{\sum (X_i - \bar{X})^2}{n-1} = \frac{16.22}{11} = 1.47$$

$$5 \text{ Standard deviation } - s = \sqrt{\frac{\sum X_i^2 - \frac{(\sum X_i)^2}{n}}{n-1}}$$

$\bar{n}$	X_i	nX_i	X_i^2	nX_i^2
1	98	98	9604	9604
2	99	198	9801	19602
4	100	400	10000	40000
3	101	303	10201	30603
2	102	204	10404	20808
		1203		120617

$$s = \sqrt{\frac{120617 - \frac{1203^2}{11}}{11}} = \sqrt{\frac{120617 - 120601}{11}}$$

$$s = \sqrt{\frac{16}{11}} = 1.21$$

6 Aid in calculation

Recalling that adding or subtracting a constant to each score in the distribution doesn't affect the variance or the standard deviation of the distribution we can simplify the computations by first subtracting 100 from each score in the distribution, thus obtaining the frequency distribution shown in Table 2.

$\bar{n}$	$X_i - C$	$n(X_i - C)$	$(X_i - C)^2$	$n(X_i - C)^2$
1	-2	-2	4	4
2	-1	-2	1	2
4	0	0	0	0
3	1	3	1	3
2	2	4	4	8
		3		17

$$s^2 = \frac{\sum X_i^2 - \frac{(\sum X_i)^2}{n}}{n-1}$$

$$s^2 = \frac{17 - \frac{(3)^2}{11}}{11} = \frac{16.25}{11} = 1.48$$

$$s = \sqrt{\frac{\sum X_i^2 - \frac{(\sum X_i)^2}{n}}{n-1}} = \sqrt{1.48} = 1.22$$

7 Standard deviation of the mean -

$$S = \frac{s}{\sqrt{n}}$$

Using calculations from number 6,

$$S = \frac{s}{\sqrt{n}} = \frac{1.22}{\sqrt{12}} = \frac{1.22}{3.46} = 0.35$$

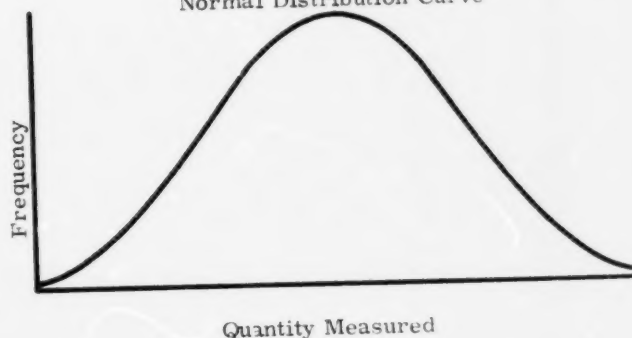
8 Relative standard deviation expressed as a percent (coefficient of variation)

$$V = \frac{s}{\bar{X}} \times 100$$

Using calculations from number 6 for $s = 1.22$ and from number 2 for $\bar{X} = 100.25$,

$$V = \frac{s}{\bar{X}} \times 100 = \frac{1.22}{100.25} \times 100 = 1.21\%$$

Figure 2
Normal Distribution Curve



V INTRODUCTION TO NORMAL DISTRIBUTION CURVE

- A Statistics deals with theoretical curves which are smoother than frequency polygons, obtained from experiments in real life. However, frequency distributions or frequency polygons of experimental data often approximate a mathematical function called the "normal" distribution curve. (See Figure 2)

As shown in Figure 3, the frequency polygon for the 12 determinations for chloride in water is a fairly good approximation of the normal curve. If, however, in the chloride determinations we had obtained 103 instead of 98 and 104 instead of 99 this distribution would not have been a good approximation of the normal curve, as is shown in Figure 4.

Figure 3

Comparison of Normal Curve and Frequency Polygon

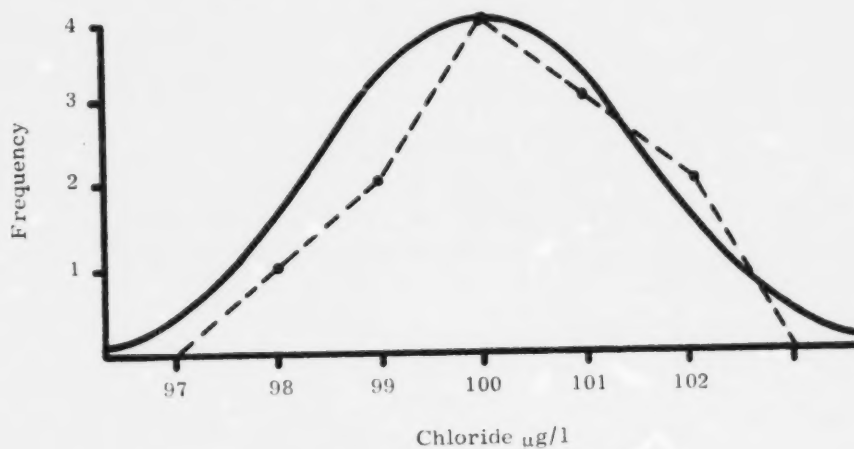
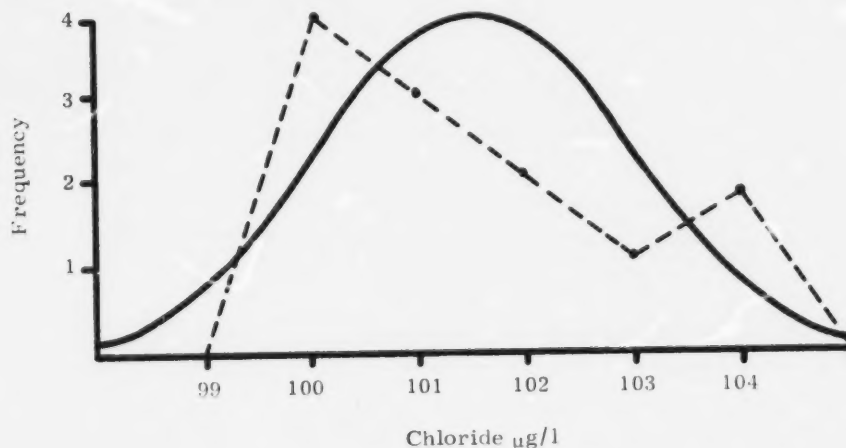


Figure 4

Comparison of Normal Curve and Frequency Polygon



B If a frequency distribution is a good approximation of the normal curve, we can use some facts about the normal curve to give us information about the frequency distribution.

Figure 5 shows the normal distribution in terms of the population mean μ , and the standard deviation of the population σ , and gives the percent of area under the curve between certain points.

Figure 5
Normal Distribution Curve

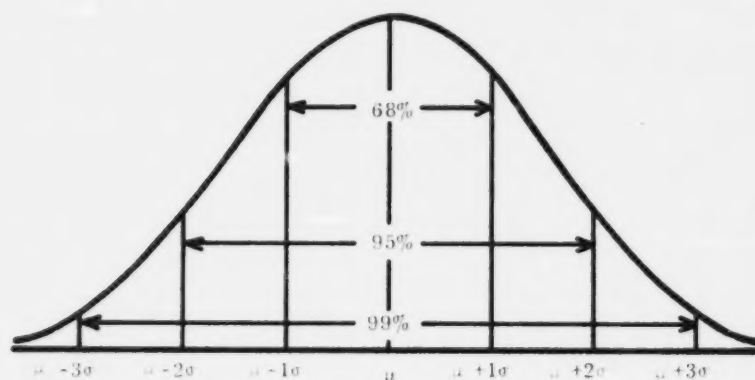
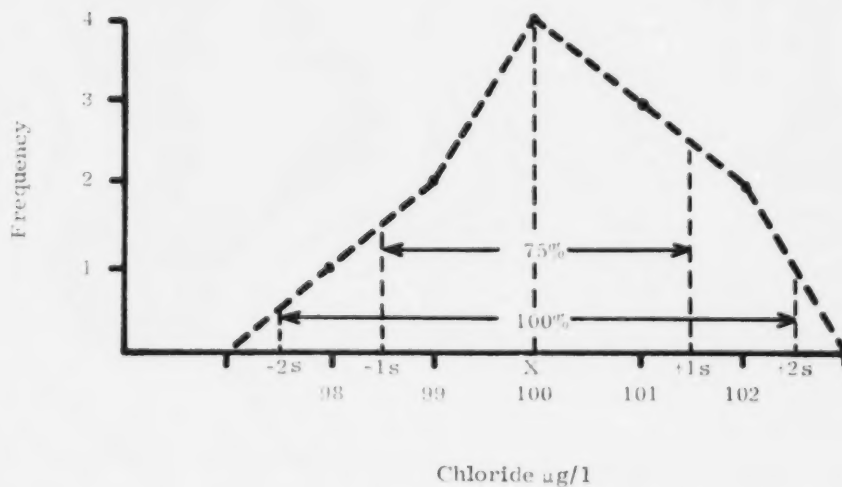


Figure 6
Frequency Distribution Polygon



We may check the distribution of sample data to see if it is a "normal" distribution in the following manner. Substitute the value of the sample mean ($\bar{X}$) for the value of the midline and substitute the value of the sample standard deviation (s) for the limits of the value spans where we might expect certain percentages of the data items to occur. Then we can check the number of data items which actually do occur within these value spans.

Figure 6 demonstrates this application using the chloride data values from Table 1. The data values are marked on the horizontal line and the frequency of the occurrence of each value is marked on the vertical. The midline of the distribution is marked at the value of the sample mean ($\bar{X} = 100$, See III C 3). The value of the sample standard deviation ($s = 1.21$, See IV C 5) is used to mark value areas under the curve where different percentages of data values will probably occur. Thus, for the area $\bar{X} \pm 1s$, $\bar{X} - 1s = 98.79$ and $\bar{X} + 1s = 101.21$. Therefore, according to the normal distribution curve shown in Figure 5, we might expect about 68% of the data items to have values between 99 and 101. (The values are rounded to whole numbers since the data values are thus recorded).

Consulting Table 1, we find that 75% or 9 of the 12 data items have values in this range. This percentage is shown in Figure 6 by the frequency polygon for the data shown earlier in Figure 3.

Likewise assuming a normal distribution, we would expect 95% of the observations to lie within $\pm 2s$'s from the population mean. In fact, 100% of the observations were within $\pm 2s$'s from the sample mean.

In both cases the observed percentages are reasonably close to the expected percentages. Other tests exist for determining whether or not a frequency distribution might reasonably be assumed to approximate the normal distribution.

It would be good to become as familiar as possible with the normal distribution since an underlying normal distribution is assumed for many statistical tests of hypothesis.

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This outline was prepared by L. A. Lederer, Statistician, formerly with Analytical Reference Service, Training Program, NCUH, SEC. Revised by Audrey E. Donahue, Chemist, National Training Center, Water Programs Operations, EPA, Cincinnati, OH 45268.

ACCURACY-PRECISION-ERROR

I INTRODUCTION

An analytical method is subject to errors. These errors may affect the accuracy of the method because they introduce bias into the results. There are other types of errors which affect the precision of the method because they produce random fluctuations in the data. The most desirable situation for the analyst is shown in the diagram in Figure 1 where the results are both precise and accurate.

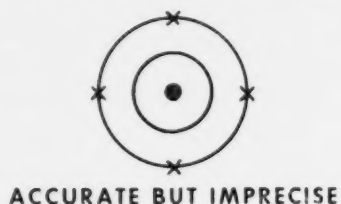
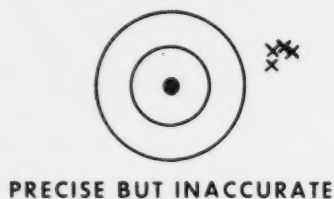


Figure 1 PRECISION AND ACCURACY

A Accuracy

For results to be accurate, the analysis used must give values close to the true value. (See Figure 1)

B Precision

Precision is the degree of agreement among results obtained by repeated measurements on a single sample under a given set of conditions. It is a measure of the degree to which results "check." (See Figure 1)

C Note

It is possible to have precision without accuracy. (See Figure 1)

II DETERMINATE ERROR AND ACCURACY

A determinate error is one which contributes a constant error or bias to results, causing them to be inaccurate. This constant error makes it possible for results to agree with each other (be precise) and still be inaccurate. (See Figure 1)

Determinate errors have "assignable" causes which can usually be identified and either eliminated or controlled. (The terms "determinate" error, "assignable" error, and "systematic" error are synonymous).

A Sources of Determinate Error

1 Method error

Method errors are those that are inherent in the procedure. These are very serious and the hardest to detect and correct. The most common method error is the presence of interferences in the sample. Other examples would be precipitation of substances other than the desired material, partial solubility of precipitates, and entrainment as in a solvent extraction procedure.

2 Personal errors

Personal errors are attributable to individual mistakes which are consistently made by an analyst. These errors are the result of consistent carelessness, lack of knowledge or personal bias. Examples are errors in calculations, use of contaminated reagents, non-representative sampling, or poor calibration of standards and instruments.

3 Instrumental errors

Instrumental errors are those which are caused by an analytical instrument or by the effects of the environment acting on the instrument. Moisture in a G.C. column, improper wavelength markings on a spectrophotometer or incorrect scoring on a buret would be examples.

B Effects of Determinate Error

1 Additive

An additive determinate error is one which has a constant value regardless of the amount of analytically sought constituent present in the sample. (See Figure 2)

2 Proportional

A proportional determinate error changes value according to the amount of analytically sought constituent in the sample. (See Figure 3)

III DETECTION OF DETERMINATE ERROR

A Spiked Samples

- 1 Samples which can be determined before and after the addition of a known constituent (in the concentration range of interest) provide a way to detect determinate errors. Spiked samples should be representative and resemble actual conditions as closely as possible. The quantitation of bias can then be obtained with the following measures.

- a Mean error - the difference between the mean of the data and the true result.

- b Relative error - the mean error of a set of data expressed as a percentage of the true result.

EXAMPLE: An analyst determines the nitrate content of the effluent from his sewage treatment plant to be 0.5 mg/l. He then adds 1 mg/l of standard nitrate solution to the sample. Table 1 shows the replicate results obtained on the spiked sample, and calculation of both mean and relative errors.

Table 1

Sample: Effluent
Determination: Nitrate (Modified Brucine)

X_i

1.55 mg/l

1.58

1.60

1.47

1.35

1 Calculation of mean error

$$\bar{X} = \frac{7.55}{5} = 1.51 \text{ mg/l}$$

$$\text{Mean error} = +0.01 \text{ mg/l}$$

2 Calculation of relative error

$$\text{Relative Error} = \frac{0.01 \times 100}{1.50} = 0.7\%$$

2 Control charts

Trends and shifts on control charts may also indicate determinant error. Using spiked samples, the standard deviation is calculated and control limits (usually ± 3 standard deviations) for the analysis are set (see Figure 4). For further discussion of control limits, see reference 3, p. 62.

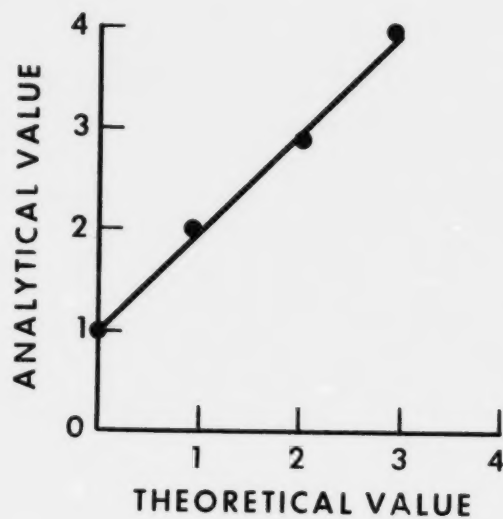


Figure 2. ADDITIVE ERROR

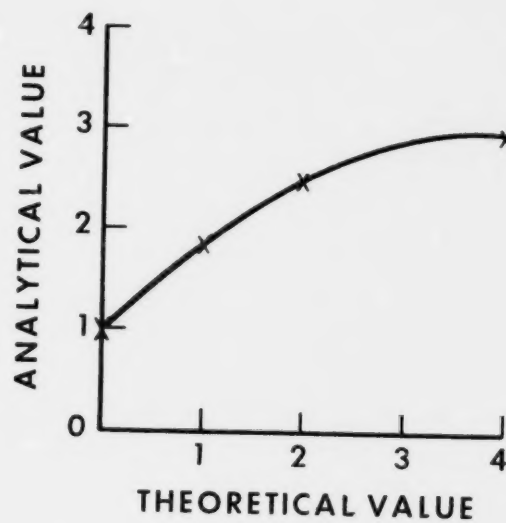


Figure 3. PROPORTIONAL ERROR

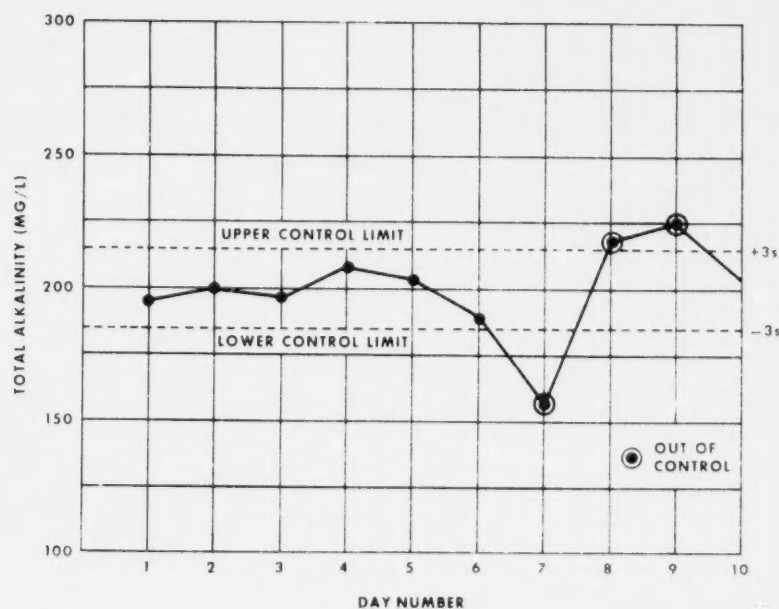


Figure 4. CONTROL CHART

- 3 In some cases, it is impossible to spike a sample so that it resembles actual conditions (e.g., BOD and pesticide samples). Youden (11) provides excellent techniques for detecting bias in this situation.

B Unknown Samples

1 Independent method

Analysis of a sample for a desired constituent by two or more methods that are entirely different in principle (gravimetric and volumetric) may aid in the estimation of determinate error. However, another reliable method may not be available or may be laborious to perform.

2 Control charts

It is possible to plot a control chart (Figure 4) even when it is not possible to spike a sample. One can use as a reference value an average of a series of replicate determinations performed on a composite check sample. Such a sample must be preserved or stabilized in such a way that the concentration of the constituent being measured will not change from day to day (see reference 1).

3 Aliquoting

If the determinate error is additive, the magnitude may be estimated by plotting the measured quantity versus a range of sample volumes or sample weights. If the error has a constant value regardless of the amount of analytically sought constituent, then a straight line fitted to the points will not go through the origin. (See Figure 5)

C Youden's Graphical Technique (10, 11, 12)

Dr. W. J. Youden has devised an approach to test for determinant errors with a minimum of effort on the part of the analyst and his laboratory. Samples used may be of known (spiked) or unknown composition.

1 Technique

Two different test samples (X and Y) are prepared and distributed for analysis to as many individuals or laboratories as possible. Each participant is asked to perform only one determination on each sample (NOTE: It is important that the samples be relatively similar in concentration of the constituent being measured). Table 2 shows the results on two such samples analyzed for percent potassium by 14 different laboratories. The mean for each sample has been calculated.

2 Interpretation - Figure 6

The vertical line drawn on the graph represents the mean ($\bar{X}$) of all the results obtained on Sample X; the horizontal line was drawn through the mean ($\bar{Y}$) of all the results obtained on sample Y. Each pair of laboratory results can then be plotted as a point on the graph (marked X, 1 etc.)

If the ratio of the bias (error) to standard deviation is close to zero for the determinations submitted by the participants, then one would expect the distribution of the paired values (or points) to be close to equal among the four quadrants. The fact that the majority of the points fall in the (+, +) and (-, -) quadrants indicates that the results have been influenced by some source of bias or determinate error.

Furthermore, one can even learn something about a participant's precision from the graph. If all participants had perfect precision (no indeterminate error), then all the paired points would fall on a 45° line passing through the origin. Consequently the distance from such a 45° line of each participant's point provides an indication of that participant's precision.

- 3 The Youden method is also discussed in this manual in the section on Interlaboratory Quality Control Studies. A quantitative treatment of this subject can be found in references 11 and 12.

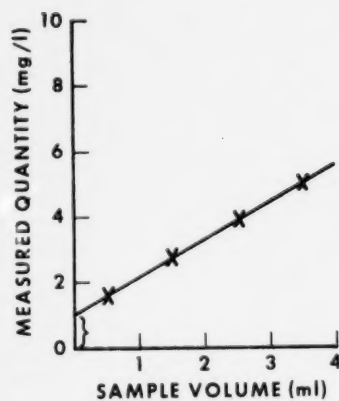


Figure 5

Table 2

Laboratory	Sample X	Sample Y
1	9.74%	8.50%
2	9.92	8.28
3	9.98	8.84
4	9.99	8.24
5	10.00	8.73
6	10.11	8.54
7	10.12	8.64
8	10.14	8.82
9	10.19	9.04
10	10.23	8.93
11	10.25	8.97
12	10.29	8.80
13	10.55	9.21
14	10.62	8.95

$$\bar{X} = 10.15 \quad \bar{Y} = 8.75$$

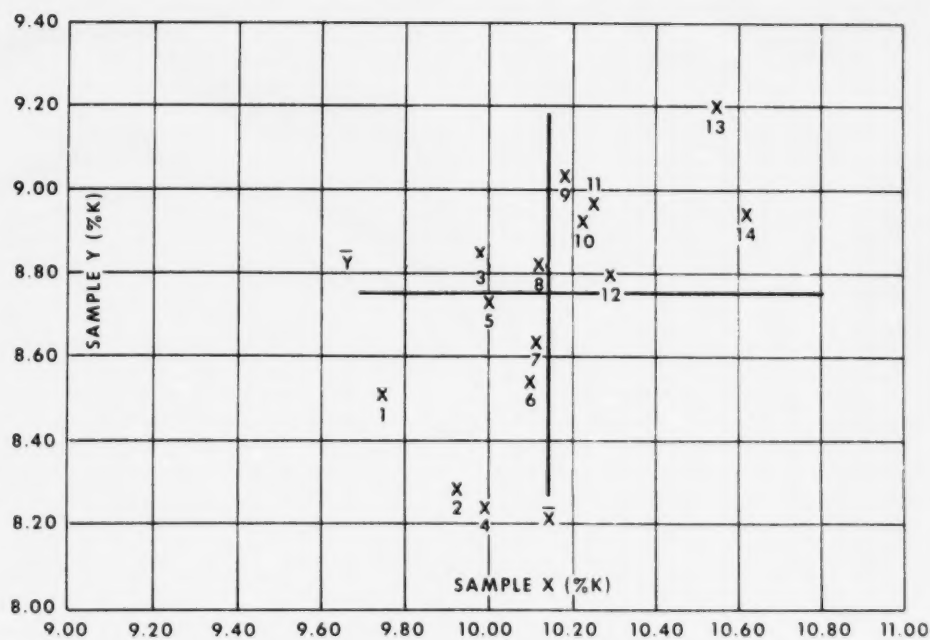


Figure 6. YOUDEEN'S GRAPHICAL TECHNIQUE

IV ELIMINATION OF DETERMINATE ERROR

Various approaches can be used to eliminate the source of determinate error. The approach used depends upon whether the source is personal, method, or instrumental.

A Personal

Great care must be used to avoid producing an unconfident attitude in a technician. It is undesirable for the analyst to feel he is being "policed."

B Method and Instrumental

1 Blanks

Blanks can be used to correct for interferences from reagents, sample color, etc.

2 Correction factors

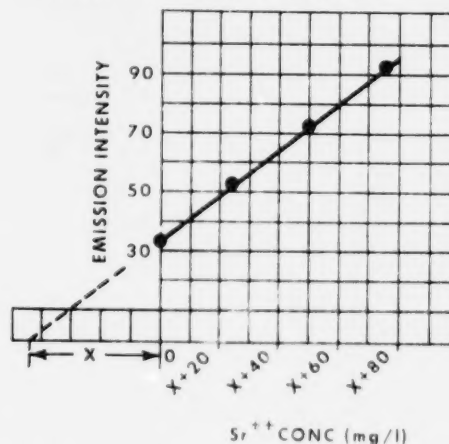
Examples of correction factors used in environmental analyses to eliminate determinate errors are the following:

- a Recovery factors in organic extractions
- b Chemical yield values in gravimetric analyses
- c Counting efficiencies for radiation counters

3 Standard addition

Sample interferences producing determinate errors can be overcome by adding equal amounts of unknown sample to a series of standards. The concentration of the unknown can then be determined graphically from a plot of the measured quantity (absorption, emission, etc.) versus the standard concentration. (See Figure 7)

Figure 7. METHOD OF STANDARD ADDITION GRAPHICAL METHOD OF COMPUTING Sr^{++} CONCENTRATION



NOTE: The X value appearing along the abscissa (i.e., $X + 20$, $X + 40$, etc.) refers to the unknown added to the strontium standard

4 Standard compensation

Another approach is to prepare the standard so that its composition resembles that of the sample as closely as possible. The objective of the approach is exactly the same as that of standard addition - to compensate for the presence of interfering substances in the unknown.

V INDETERMINATE ERROR AND PRECISION

Even when all determinate errors are eliminated, every replicate analysis will not give the same value. Such variation in results is due to indeterminate error, also known as random, chance or uncontrollable error. Indeterminate error affects the precision or agreement among results.

A Sources of Indeterminate Error

Indeterminate errors are due to unassignable or "chance causes." Examples are inadvertent contamination of sample or glassware, variation in reagent additions, or variations in instrument response (see Figure 8).

B Effects of Indeterminate Error

Since the causes of these errors are random, the effects are also random. Fortunately, these random variations conform to the "Laws of Chance" so statistical measures of precision can be used to quantitate indeterminate errors.

VI DETECTION OF INDETERMINATE ERROR

A measure of the degree of agreement among results can be obtained by analyzing a single sample repeatedly under a given set of conditions.

A Range

The range of the replicate results (difference between the lowest and the highest value) provides a measure of indeterminate variations.

B Standard Deviation

An estimation of indeterminate error can be obtained through a calculation of the standard deviation. The following formulas should be applied to random data which follows a normal distribution. Normality can be checked by ranking and plotting the data on normal probability paper; it should fall on a straight line (see Figure 9). Any values which do not fall close to the straight

line (those values encircled) should be rejected in the calculation of standard deviation. Other statistical tests (8) can be used to objectively evaluate the rejection of outliers. The value obtained for standard deviation for a particular method may vary with the analyst, the concentration range of the constituent, and the composition of the sample analyzed. The confidence of the estimate is increased as the number of results (n) used to compute the standard deviation is increased.

1 Replicate results on the same sample:

$$s = \sqrt{\frac{\sum (X_i - \bar{X})^2}{n-1}} \quad (1)$$

X_i = value of single result

$\bar{X}$ = average (mean) of results on same sample

n = number of results

Example: Table 3 contains a set of 5-day BOD results obtained on a synthetic sample containing 150 mg/l of glucose and 150 mg/l glutamic acid. (Note: A 1% dilution was used in the actual test). The results are those submitted by laboratories participating in a collaborative study. Calculate the standard deviation of these results.

$$\bar{X} = 192 \text{ mg/l}$$

$$n = 36.0$$

$$\sum (X_i - \bar{X})^2 = 58,200$$

$$s = \sqrt{\frac{58,200}{35}}$$

$$s = 41 \text{ mg/l}$$

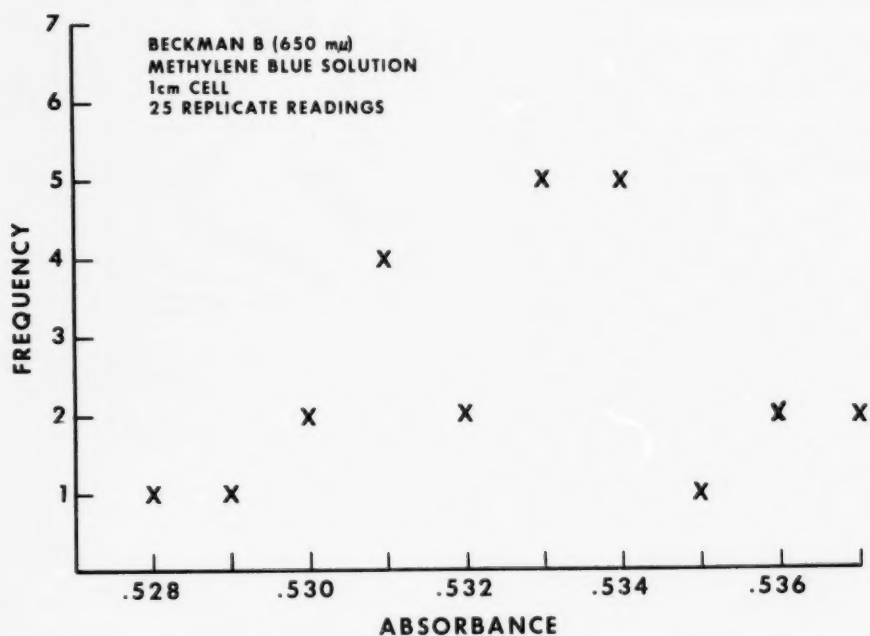


Figure 8. VARIATIONS IN SPECTROPHOTOMETER READINGS

FIGURE 9 NORMAL PROBABILITY CURVE

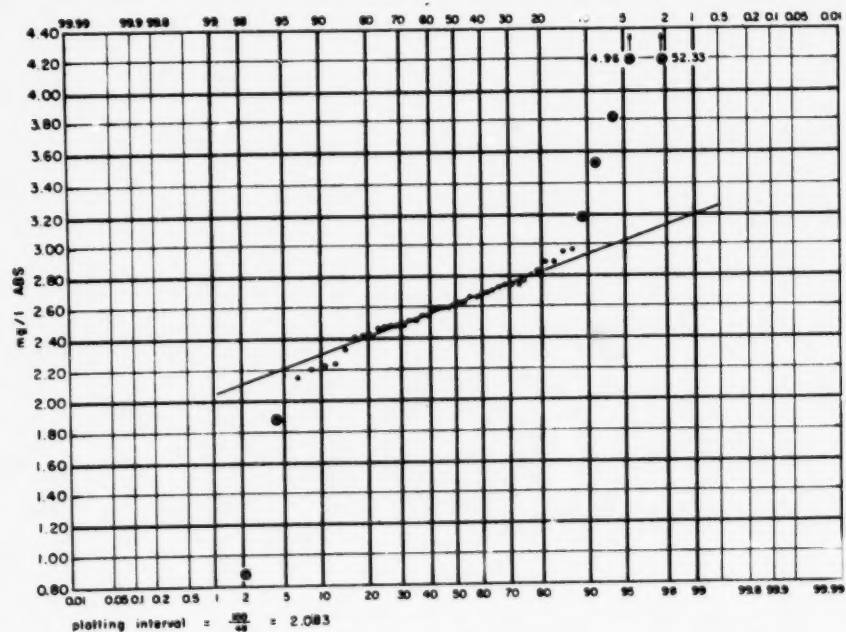


Table 3

SAMPLE: 150 mg/l glucose + 150 mg/l glutamic acid (1% dilution)
 DETERMINATION: 5-day Biochemical Oxygen Demand

X_i	$X_i - \bar{X}$	$(X_i - \bar{X})^2$	X_i	$X_i - \bar{X}$	$(X_i - \bar{X})^2$
100 mg/l	-92 mg/l	8464 (mg/l) ²	198 mg/l	6 mg/l	36 (mg/l) ²
117	-75	5625	199	7	49
125	-67	4489	200	8	64
132	-60	3600	200	8	64
142	-50	2500	204	12	144
147	-45	2025	210	18	326
153	-39	1521	211	19	361
160	-32	1024	212	20	400
165	-27	729	215	23	529
165	-27	729	223	31	961
167	-25	625	224	32	1024
173	-19	361	227	35	1225
178	-14	196	229	37	1369
189	-3	9	238	46	2116
190	-2	4	247	55	3025
196	4	16	250	58	3344
196	4	16	259	67	4489
197	5	25	274	82	6724

*Data taken from Water, Oxygen Demand Report (July, 1960), Analytical Reference Service, Training Program, R. A. Taft Sanitary Engineering Center, Cincinnati, Ohio.

Table 4*

CONCENTRATION

SAMPLE: Aqueous

RANGE: 30 - 70 mg/l

DETERMINATION: Phosphate (Lucerna, Cerde, and Prat Method)

Sample	Mg/l P	d	d ²	Sample	Mg/l P	d	d ²
A	51	2	4	L	54	2	4
	53				56		
B	39	0	0	M	38	1	1
	39				37		
C	53	1	1	N	52	0	0
	54				52		
D	47	0	0	O	58	0	0
	47				58		
E	50	1	1	P	54	0	0
	51				54		
F	48	1	1	Q	54	0	0
	47				54		
G	50	0	0	R	48	0	0
	50				48		
H	47	0	0	S	52	1	1
	47				51		
I	42	0	0	T	53	0	0
	42				53		
J	50	1	1	U	46	1	1
	51				47		
K	59	1	1	V	42	0	0
	60				42		

*Data obtained from Frank Schickner, Proctor and Gamble Company.

2 Duplicate results on different samples

In a laboratory where duplicates are routinely run, it would be simple to use the following formula for evaluation of standard deviation.

$$s = \sqrt{\frac{\Sigma(d^2)}{2k}} \quad (\text{See reference 5, page 654}) \quad (2)$$

d = difference between duplicates

k = number of samples

Example: Table 4 contains a set of phosphate results obtained on aqueous samples in the range 100-200 mg/l. Calculate the standard deviation of these results.

$$\Sigma(d^2) = 16$$

$$k = 22$$

$$s = \sqrt{\frac{16}{44}}$$

$$s = .61 \text{ mg/l}$$

3 Duplicate and triplicate results on different samples

$$s = \sqrt{\frac{\Sigma(X_i - \bar{X})^2}{n - k}} \quad (\text{See reference 7, page 73}) \quad (3)$$

Note: $\bar{X}$ = average of results on the same sample

Example: Table 5 contains a set of % nitrogen results obtained on unknown organic compounds.

Calculate the standard deviation.

$$\Sigma(X_i - \bar{X})^2 = .2401$$

$$n - k = 22$$

$$s = \sqrt{\frac{.2401}{22}}$$

$$s = .104\%$$

4 Use of range to estimate standard deviation

For a small number of replicates ($n < 10$), the range can be used to estimate the standard deviation (See Table 6).

$$\frac{R}{d_N} = s \quad (4)$$

Example: Table 7 contains a set of replicate nitrate results. Calculate the standard deviation of these results.

a Use of formula (1)

$$\bar{X} = 0.72 \text{ mg/l}$$

$$n = 5$$

$$\Sigma(X_i - \bar{X})^2 = .0134$$

$$s = \sqrt{\frac{.0134}{4}}$$

$$s = .058 \text{ mg/l}$$

b Use of formula (4)

$$R = .14 \text{ mg/l}$$

$$d_N = 2.33$$

$$s = \frac{.14 \text{ mg/l}}{2.33}$$

$$s = .060 \text{ mg/l}$$

C Coefficient of Variation

An estimation of indeterminate error can also be made by calculating the coefficient of variation.

$$V = \frac{s}{\bar{X}} \times 100$$

By comparing the standard deviation (dispersion) to the average or mean value (central tendency) in a set of data and expressing this relative standard deviation as a percentage, the analyst has a meaningful interpretation of the degree of dispersion present. Indeter-

Accuracy-Precision-Error

Table 5*

SAMPLE: Unknown Organic Compounds					CONCENTRATION RANGE: 10% - 20%				
DETERMINATION: % Nitrogen (Kjeldahl)									
Sample	%N	$\bar{X}$	$X_i - \bar{X}$	$(X_i - \bar{X})^2$	Sample	%N	$\bar{X}$	$X_i - \bar{X}$	$(X_i - \bar{X})^2$
A	16.48	16.47	.01	.0001	J	13.93	13.75	.18	.0324
	16.46		.01	.0001		13.56		.19	.0361
B	16.50	16.50	0	0	K	10.34	10.27	.07	.0049
	16.49		.01	.0001		10.19		.08	.0064
C	16.72	16.65	.07	.0049	L	17.16	17.15	.01	.0001
	16.57		.08	.0064		17.13		.02	.0004
D	17.52	17.58	.06	.0036	M	15.01	15.03	.02	.0004
	17.60		.02	.0004		15.05		.02	.0004
	17.63		.05	.0025	N	12.44	12.62	.18	.0324
E	16.31	16.31	0	0		12.70		.08	.0064
	16.30		.01	.0001		12.73		.11	.0121
F	16.40	16.35	.05	.0025	O	14.37	14.37	0	0
	16.21		.04	.0016		14.36		.01	.0001
	16.35		0	0	P	11.85	11.85	0	0
G	17.56	17.55	.01	.0001		11.85		0	0
	17.54		.01	.0001	Q	14.79	14.73	.06	.0036
H	14.96	14.81	.15	.0225		14.70		.03	.0009
	14.66		.15	.0225		14.70		.03	.0009
I	19.15	19.02	.13	.0169	R	17.19	17.17	.02	.0004
	18.89		.13	.0169		17.14		.03	.0009

*Data obtained from Frank Schickner, Proctor and Gamble Company.

Table 6*

FACTORS USED TO ESTIMATE THE STANDARD DEVIATION FROM RANGE

Size of Sample (n)	d_N	$\frac{1}{d_N}$
2	1.13	.887
3	1.69	.591
4	2.06	.486
5	2.33	.430
6	2.53	.395
7	2.70	.370
8	2.85	.351
9	2.97	.337
10	3.08	.325

(8)
*Natrella, Experimental Statistics, pp. 2-6.

Table 7

SAMPLE: Ohio River Water

DETERMINATION: Nitrate (Modified Brucine)

X_i	$X_i - \bar{X}$	$(X_i - \bar{X})^2$
0.65 mg/lN	-0.07	.0049
0.68	-0.04	.0016
0.70	-0.02	.0004
0.76	+0.04	.0016
0.79	+0.07	.0049

minate error would be the cause of undue dispersion.

Example: Table 3

$$s = 41 \text{ mg/l}$$

$$\bar{X} = 192 \text{ mg/l}$$

$$V = \frac{41}{192} \times 100$$

$$V = 21\%$$

- D An indication of an individual's analytical precision can be obtained by his participation in an interlaboratory study. In this case, the analyst performs only one determination on each of two samples. Using Youden's Graphical Technique as an indication of an individual's precision was discussed previously in this outline in III C. Also see the outline on "Interlaboratory Quality Control Studies."

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This outline was prepared by Betty A. Punthorst, Chemist formerly with National Training Center, and revised by Audrey E. Donahue, Chemist, National Training Center, MDS, WPQ EPA, Cincinnati, OH 45268.

INTERLABORATORY QUALITY CONTROL STUDIES

I DEFINITION OF INTERLABORATORY STUDY IN EPA

- A It is a between-laboratory evaluation of exact physical, chemical or biological methods which yield a measurement of desired properties.
- B It is not an interlaboratory evaluation of materials, reagents, or different test conditions. It is not an initial study of a method, nor a study to develop methods.

II PURPOSES OF INTERLABORATORY STUDY

- A Selection of an analytical method.
- B Evaluation of an analytical method.
- C Evaluation of laboratory and analyst performance.

III PREPARATION FOR INTERLABORATORY STUDY

- A Laboratories are in control. Between-replicate deviation will then be very small and uniform in all laboratories.
- B Ruggedness testing of a method has been completed by the method developer, or by a single qualified laboratory. Effects of small changes in time, temperature, pH, reagent and sample volume measurements, etc., are known and corrected for.⁽¹⁾
- C All laboratories and analysts are familiar with the method to be tested. Any special equipment or reagents required are known and available. If all analysts are not acquainted with the method, a simple preliminary study is undertaken to accomplish this.⁽²⁾

IV INSTRUCTIONS FOR THE STUDY

- A Exact method write-ups are provided to each analyst. The technical description of an analytical method is difficult. The language and organization must be complete, yet simple and unambiguous. These characteristics are often conflicting.

Requirements in a method may seem trivial. Yet if these are not recognized and controlled, the entire study can be useless.

- B Explicit directions for sample preservation, sample make-up, time limitations, sequence of analyses, etc., are provided.
- C Advance notice of tests is given so that laboratories can integrate the test into their program and realistic deadlines can be established. Once established, deadlines for agreement to participate, for completion of analyses and for reporting are followed closely.

V THE TEST SAMPLES

- A The sample is carefully designed to reflect the concentrations found in natural waters and yet be within the best portion of the concentration range for the method.
- B Since precision of almost all methods varies with concentration, a comprehensive study includes several levels of concentration.
- C Since accuracy of a method is very important, exactly known levels of constituents are added both to distilled and natural waters for testing. Stabilized natural waters are not used as samples.

VI SAMPLE FORM AND CONTAINER

A In EPA, Research and Monitoring Office, samples are prepared as concentrates for final dilution at the testing laboratory. Use of concentrates has several advantages. This greatly reduces space requirements and thereby greatly reduces cost of mailing. With use of sealed glass ampules, preservation of concentrates is easily obtained through steam sterilization. Chemical preservatives can be used at fairly high levels in the concentrate and can be removed as interferences by the dilution of the concentrate to final sample volume. Theoretically, a well designed sample properly sealed in glass is stable indefinitely.

B Samples can be prepared as dilute simulated or natural water samples to be analyzed, as is. These samples have an advantage in that they reach the analyst exactly as a routine sample. There is no error in making a dilution and this source of variance in the method is removed.

Some disadvantages of such samples are: the serious logistics problem from storage if a large number of samples is involved; a relatively high cost of mailing; and a limited choice of preservation methods because of the size of containers, the probable use of plastic containers, and only limited testing of water types.

VII INTERLABORATORY STUDY DESIGN

- A Sample concentrates are prepared in similar yet different concentrations at each of several levels of analysis.
- B True values are calculated from the amount of a constituent added, and are used to measure the accuracy of the study method.
- C Multiple constituents are prepared in a sample by related groups of analyses.

For example, a mineral and physical sample is analyzed for pH, alkalinity/acidity, specific conductance, total hardness, sulfate, chloride, total solids, calcium, magnesium, sodium and potassium. A nutrient sample is analyzed for ammonia nitrogen, nitrate nitrogen, Kjeldahl nitrogen, orthophosphate and total phosphorus.

- D The analyst dilutes separate aliquots to volume with distilled water and with natural water of his choice.
- E Single analyses are made for each parameter. Replicates are of limited value in evaluating a method for routine analyses because of data handling problems and failure to detect significant differences by replication.
- F Recoveries are compared for distilled and for natural waters. Recovery from distilled water should indicate method bias. Differences in recoveries from distilled and natural waters should indicate interference by natural water samples.

VIII DATA EVALUATION AND REPORTING

A Rejection of Outliers-Accuracy.

The T-Test is applied to all data using the standard deviation of all data and compared with a T value at the 99% confidence interval. Accuracy is calculated after rejection of this data.

B Precision Measures

A statistical summary is developed for computerized treatment of data as suggested by Larsen⁽³⁾. Method precision is reported as standard deviation, 95% confidence interval, and coefficient of variation. Method precision is reported for each sample since it varies with concentration.

C Graphic Display

Methods are evaluated using two-sample charts described by Youden⁽⁴⁾. The values reported by an analyst for a sample pair became the x and y coordinates for a single data point on the chart. Data form ellipses around the true value because of systematic error. Random error is shown as the perpendicular distance from the 45° slope. General scatter indicates poor method precision. Bias is shown by movement of the ellipse away from the true values. Outliers are visible at some distance from the major grouping of data. Examples shown on the following pages are:

Figure 1 - Outliers,

Figure 2 - Limited precision and limited accuracy,

Figure 3 - Negative bias,

Figure 4 - Positive bias,

Figure 5 - Systematic error,

Figure 6 - Good precision and accuracy.

IX INTERLABORATORY STUDY REPORT^(5,6)

A Reports are slanted toward audience.

B All data are coded by laboratory and analyst.

C Glossary of terms is necessary.

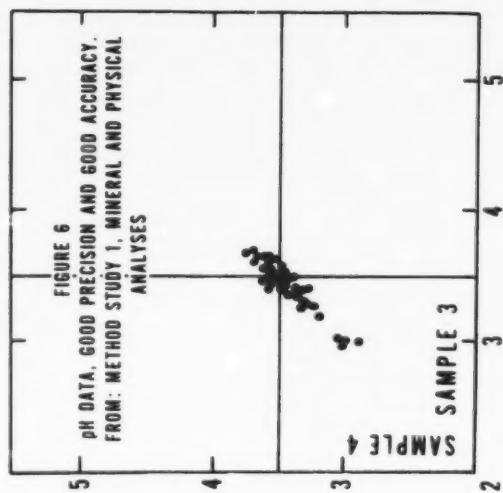
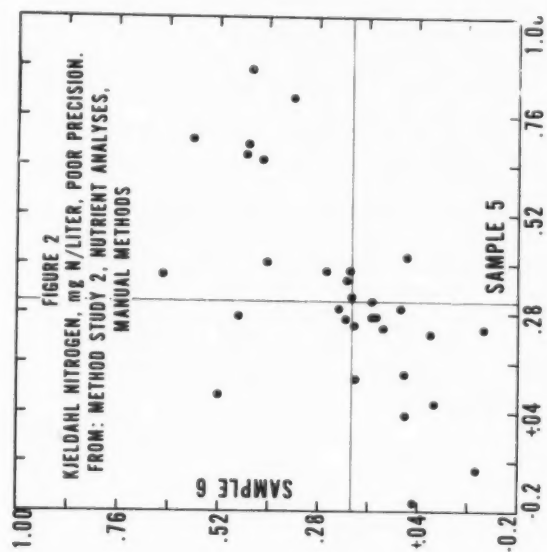
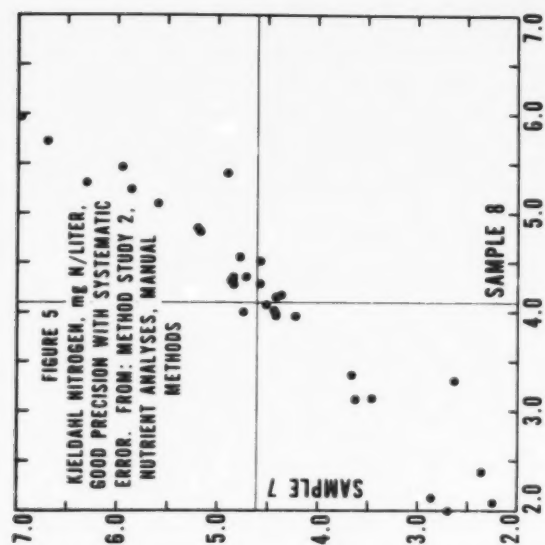
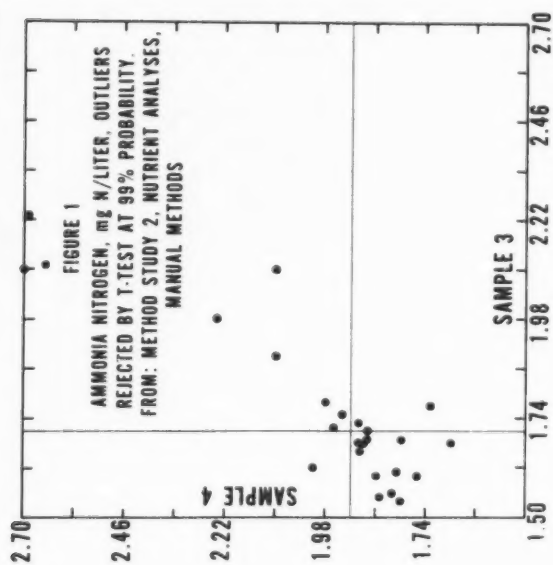
D Computer use increases statistical capabilities tremendously. There is a danger of "over-evaluation".

E Increased efficiency in reporting data is achieved by using the computer print-out directly in a report. See Figures 7 and 8.

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This outline was prepared by J. A. Winter, Chief, Method & Performance Evaluation, Analytical Quality Control Laboratory, National Environmental Research Center, EPA, Cincinnati, Ohio.



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This outline was prepared by J. A. Winter, Chief, Method & Performance Evaluation, Analytical Quality Control Laboratory, National Environmental Research Center, EPA, Cincinnati, Ohio.

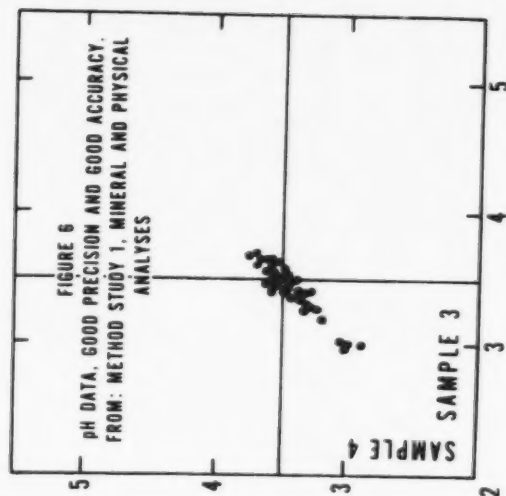
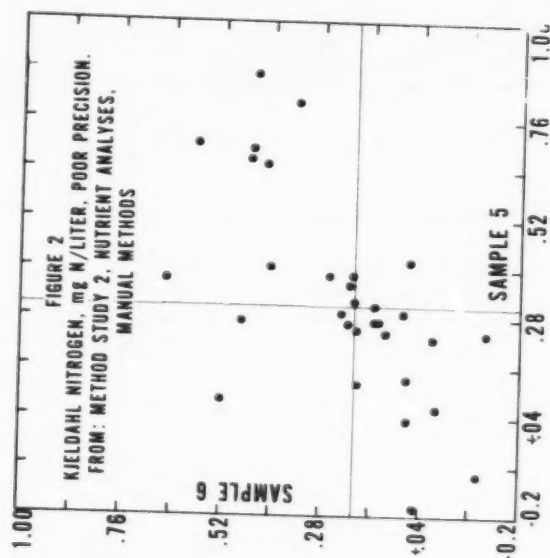
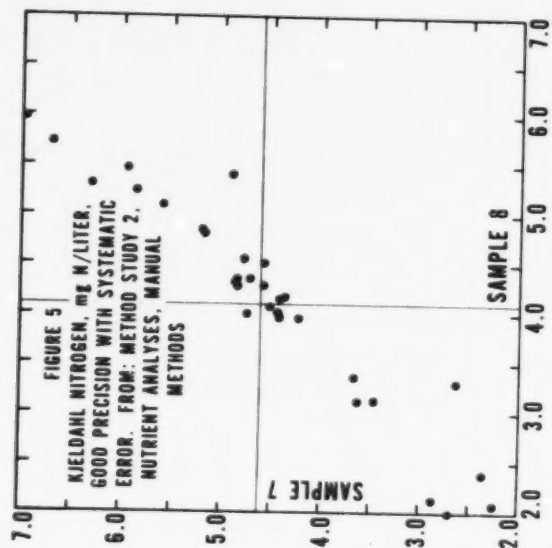
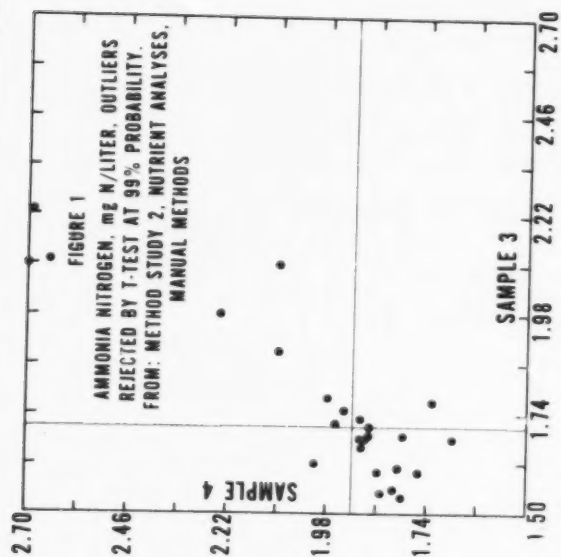
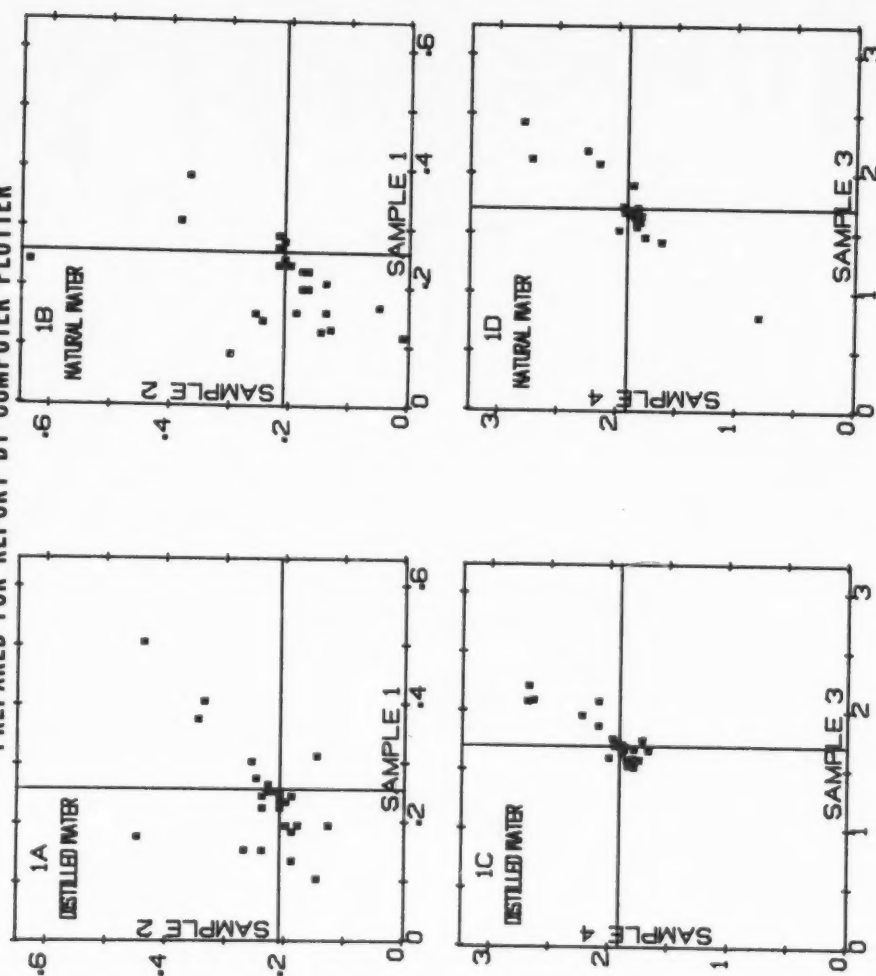


FIGURE 8
AMMONIA NITROGEN DATA, YODEN'S TWO-SAMPLE CHARTS.
PREPARED FOR REPORT BY COMPUTER PLOTTER



III. STANDARD SOLUTIONS, ACIDITY, ALKALINITY

	<u>Outline Number</u>
Analytical Reactions - Standard Solutions	6
Acidity, Alkalinity, pH and Buffers	7
Alkalinity and Relationships Among the Various Types of Alkalinities	8

ANALYTICAL REACTIONS - STANDARD SOLUTIONS

I INTRODUCTION

- A A standard solution is one whose composition and concentration are known to a high degree of accuracy. In chemical analyses, it is used to determine the concentration of a particular component in a sample.
- B These chemical analyses very frequently involve an acid-base reaction or an oxidation-reduction reaction.
- C Three important terms connected with these two types of chemical reactions are: mole, equivalent weight, and normality. These terms will be defined in following sections.

II ANALYTICAL CHEMICAL REACTIONS

- A As stated above, in a chemical analysis a volume of standard solution is brought into contact with a volume of sample in order to determine the concentration of some particular component of the sample.
- B For a given volume of sample, it is necessary to use a definite amount of the standard solution--too much or too little would give erroneous results.
- C For example, one cannot simply mix together random volumes of sodium hydroxide and hydrochloric acid solutions and expect the only two substances produced to be sodium chloride and water.
- D Unless the concentrations of the two reagents are known and the amounts measured accurately, excess sodium hydroxide or hydrochloric acid will also remain at the end of the reaction.
- E The reason for these limitations is that when molecules react with one another, they do so in definite ratio.

Unless the number of molecules of each reactant is known, there will always be an excess of one of the reactants remaining at the end of the chemical reaction. As mentioned before, this leads to erroneous analytical results.

- F Because of their size, it is not possible to count out numbers of molecules. However, the number of molecules in a quantity of a chemical may be found by determining its weight and consulting a table which lists the weights of the atoms making up the chemical.
- G For example, sodium hydroxide has the formula NaOH. It can also be stated that a molecule of sodium hydroxide consists of one sodium atom, one hydrogen atom and one oxygen atom.

One sodium atom weighs 23 atomic mass units (amu). An oxygen atom weighs 16 amu; and a hydrogen atom weighs 1 amu.

A molecule of sodium hydroxide, therefore, weighs 40 amu; all amu values have been rounded off. Forty amu is the molecular weight of sodium hydroxide.

- H A mole of any chemical is a number of grams numerically equal to the molecular weight of that chemical. One mole of sodium hydroxide, therefore, contains 40 grams (40 g).
- I Similarly, the atomic weight of a chlorine atom is 35 amu; that of a hydrogen atom is 1 amu; and the molecular weight of the hydrogen chloride molecule is 36 amu. One mole of hydrogen chloride weighs 36 g.
- J Synonyms for mole are: mol, gram mol, gram mole, and gram molecular weight.
- K Tables listing atomic weights of the elements can be found in virtually all

of the texts used for high school and first year college chemistry courses.

- L The remaining two terms mentioned in IC (equivalent weight and normality) will be considered as they are related to acid-base and oxidation-reduction reactions.

III ACID-BASE REACTIONS

- A Recall that an acid is a substance which donates a hydrogen ion, or proton, (H^+) in a chemical reaction and a base is a substance which donates a hydroxide ion (OH^-) in a chemical reaction. Reactions involving these two ions are termed acid-base reactions.

- B In the case of sodium hydroxide, the molecular weight is 40 amu and one mole of sodium hydroxide weighs 40 g. One hydroxide ion is contained in the sodium hydroxide molecule.

- 1 For a base, the number of grams in a mole divided by the number of hydroxide ions equals a quantity called the equivalent weight. Examples are given below. All amu values have been rounded off.

a Base - potassium hydroxide KOH

Atoms	Number	Wt/atom	Total
K	1	39 amu	39 amu
O	1	16	16
H	1	1	1
			56 amu

One mole of KOH = 56 g

Number of hydroxide ions = 1

Equivalent weight of KOH = 56 g

b Base - magnesium hydroxide $Mg(OH)_2$

Atoms	Number	Wt/atom	Total
Mg	1	24 amu	24 amu
O	2	16	32
H	2	1	2
			58 amu

One mole of $Mg(OH)_2$ = 58 g

Number of hydroxide ions = 2

Equivalent weight of $Mg(OH)_2$ = 29 g

- 2 For an acid, the number of grams in a mole divided by the number of hydrogen ions is the equivalent weight. Examples are given below. All amu values have been rounded off.

a Acid - nitric acid HNO_3

Atoms	Number	Wt/atom	Total
H	1	1 amu	1 amu
N	1	14	14
O	3	16	48
			63 amu

One mole of HNO_3 = 63 g

Number of hydrogen ions = 1

Equivalent weight of HNO_3 = 63 g

b Acid - sulfuric acid H_2SO_4

Atoms	Number	Wt/atom	Total
H	2	1 amu	2 amu
S	1	32	32
O	4	16	64
			98 amu

One mole of H_2SO_4 = 98 g

Number of hydrogen ions = 2

Equivalent weight of H_2SO_4 = 49 g

- C Normality is a method of expressing solution concentrations. If one equivalent weight of a chemical is dissolved in a solvent and the volume brought to one liter (l), the concentration of the solution is one normal (N).

- 1 The equivalent weight of KOH was calculated to be 56 g. This amount of the solid dissolved in water and diluted to a liter would give a 1 N solution.
- 2 The equivalent weight of HNO_3 was found to be 63 g. This quantity of acid diluted to a liter would give a 1 N solution.

IV OXIDATION-REDUCTION REACTIONS

- A The concepts of mole, equivalent weight and normality, as described in previous sections, apply also to oxidation-reduction reactions.

- B One definition of an oxidation is that it involves an increase in the oxidation state (charge) of an atom.

For example: $\text{FeCl}_2 \rightarrow \text{FeCl}_3$. In this conversion the Fe has been oxidized from +2 to +3.

- C A reduction is a decrease in the oxidation state (charge) of an atom.

For example: $\text{KMnO}_4 \rightarrow \text{MnO}_2$. In this conversion the Mn has been reduced from +7 to +4.

- D The equivalent weight of an oxidizing or reducing agent is calculated by dividing the number of grams in a mole of the reagent by the change in charge involved.

For example: FeCl_2 (used as a reducing agent).

Atoms	Number	Wt./atom	Total
Fe	1	56 amu	56 amu
Cl	2	70	70
			<u>126 amu</u>

One mole of $\text{FeCl}_2 = 126 \text{ g}$

Change in charge = 1

Equivalent weight of $\text{FeCl}_2 = 126 \text{ g}$

The concentration of a liter of solution which contains 126 g of FeCl_2 is 1 N.

V PRIMARY STANDARDS

- A A reagent of known purity is called a primary standard. Primary standard grade chemicals are available from chemical supply houses and the National Bureau of Standards. An accurately measured quantity of a primary standard is used for the preparation of standard solutions.

- B Other requirements of a primary standard are:

- 1 It must be stable at 105°C (the temperature used for drying).
- 2 It should not be reactive with components of the air, such as O_2 and CO_2 .

- 3 It should have a high equivalent weight so as to minimize any errors in the analysis.

- 4 It should be readily available at a reasonable cost.

VI STORAGE OF STANDARD SOLUTIONS

- A Standard solutions should be prepared using high quality distilled water.
- B Care should be taken to insure the cleanliness of the glass or plastic bottle used for storage.
- C Some solutions may decompose on exposure to light and should be stored in dark bottles.
- D The stopper or cap should fit tightly.

VII CALCULATIONS

- A The basic formula used in volumetric analysis is

$$(l \times N) \text{ of standard solution} =$$

$$(l \times N) \text{ of sample}$$

In a typical analysis, three of the four quantities are known, or found, and

$$N \text{ of sample} = \frac{(l \times N) \text{ of standard}}{l \text{ of sample}}$$

- B This formula can be rearranged to give

$$g = l \times N \times \text{equivalent weight}$$

where g and equivalent weight (ew) refer to the component being analyzed and l and N to the standard solution.

- C For example: How many g of NaOH are present in a sample if 100.0 ml of 0.2 N HCl are required for its titration?

$$g = l \times N \times \text{equivalent weight} =$$

$$\frac{100.0 \text{ ml}}{1000.0 \text{ ml/l}} \times 0.2 \times \frac{40.0}{1} = 0.8$$

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This outline was prepared by Charles R.
Feldmann, Chemist, National Training
Center, MDS, WPO, EPA, Cincinnati,
Ohio 45268.

ACIDITY, ALKALINITY, pH AND BUFFERS

I DEFINITIONS OF ACIDS AND BASES

A Arrhenius Theory of Acids and Bases (Developed about 1887)

- 1 Acid: A substance which produces, in aqueous solution, a hydrogen ion (proton), H^+ .
- 2 Base: A substance which produces, in aqueous solution, a hydroxide ion, OH^- .
- 3 The Arrhenius theory was confined to the use of water as a solvent.

B Bronsted and Lowry Theory of Acids and Bases (Developed about 1923)

- 1 Acid: A substance which donates, in chemical reaction, a hydrogen ion (proton).
- 2 Base: A substance which accepts, in chemical reaction, a hydrogen ion (proton).
- 3 Bronsted and Lowry had extended the acid-base concept to non-aqueous media; i.e., the solvent could, but did not have to be, water.

C Further development of acid-base theory dealing with electron pair donation or acceptance is unimportant.

II DEFINITIONS OF ACIDITY, ALKALINITY AND NEUTRALITY

A Acidity

A condition in which there is a preponderance of acid materials present in the water.

B Alkalinity

A condition in which there is a preponderance of alkaline (or basic) materials present in the water.

C Neutrality

- 1 It is possible to have present in the water chemically equivalent amounts of acids and bases. The water would then be described as being neutral; i.e., there is a preponderance of neither acid nor basic materials. The occurrence of such a condition would be rare.
- 2 The term "neutralization" refers to the combining of chemically equivalent amounts of acids and bases. The two products of neutralization are a salt and water.



Hydrochloric acid	Sodium Hydroxide	Sodium Chloride (a salt)
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- D The key word in the above definitions is "preponderance." It is possible to have a condition of acidity while there are basic materials present in the water, as well as conversely.

III HOW ARE DEGREES OF ACIDITY AND ALKALINITY EXPRESSED?

The pH scale is used to express various degrees of acidity and alkalinity. Values can range from 0 to 14. These two extremes are of theoretical interest and would never be encountered in a natural water or in a wastewater. pH readings from 0 to just under 7 indicate an acidic condition; from just over 7 to 14, an alkaline condition. Neutrality exists if the pH value is exactly 7. pH paper, or a pH meter, provides the most convenient method of obtaining pH readings. Some common liquids and their pH values are listed in Table 1.

TABLE 1. pH Values of Common Liquids

Household lye	13.7
Bleach	12.7
Ammonia	11.3
Milk of magnesia	10.2
Borax	9.2
Baking soda	8.3
Sea water	8.0
Blood	7.3
Distilled water	7.0
Milk	6.8
Corn	6.2
Boric acid	5.0
Orange juice	4.2
Vinegar	2.8
Lemon juice	2.2
Battery acid	0.2

5.9 - 8.4 is the common pH range for most natural waters.

IV HARD AND SOFT WATERS

In addition to being acidic or basic, water can also be described as being hard or soft.

A Hard water contains large amounts of calcium, magnesium, strontium, manganese and iron ions, relative to the amount of sodium and potassium ions present. Hard water is objectionable because it forms insoluble compounds with ordinary soap.

B Soft water contains small amounts of calcium, magnesium, strontium, manganese and iron ions, relative to the amount of sodium and potassium ions present. Soft water does not form insoluble compounds with ordinary soap.

V TITRATIONS

A The conversion of pH readings into such quantities as milligrams (mg) of acidity, alkalinity, or hardness, is not easily carried out. These values are more easily obtained by means of a titration.

B In a titration, an accurately measured volume of sample (of unknown strength) is combined with an accurately measured

volume of standard solution (of known strength) in the presence of a suitable indicator.

C The strength (called normality) of the sample is then found using the following expression:

milliliters (ml) of sample $\times$ normality (N) of sample = ml of standard solution $\times$ N of standard solution.

Three of the four quantities are known, and

N of sample = ml of standard solution $\times$ N of standard solution / ml of sample.

D In modified form, and a more specific application of the above equation, alkalinity is calculated in the following manner (12th ed. Standard Methods).

mg of alkalinity as mg CaCO_3 /liter (l)
 = ml of standard $\text{H}_2\text{SO}_4 \times \text{N}$ of standard $\text{H}_2\text{SO}_4 \times 50 \times 1000$ /ml sample.

VI INDICATORS

The term "suitable indicator" was used above. At the end of a titration, the pH of the solution will not necessarily be 7. It may be above or below 7. A suitable indicator, therefore, is one which undergoes its characteristic color change at the appropriate pH. Below are a few examples of indicators and the pH range in which they undergo their characteristic color changes. In some cases, mixed indicators may be used in order to obtain a sharper and more definite color change.

Indicator	Operational pH Range
Methyl Yellow	2.8 - 4.0
Methyl Orange	3.1 - 4.4
Methyl Red	4.4 - 6.2
Cresol Purple	7.4 - 9.0
Phenolphthalein	8.0 - 9.6
Alizarine Yellow	10.0 - 12.0

TABLE 2. pH Range of Indicators

VII BUFFERS

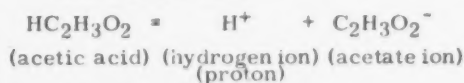
- A A buffer is a combination of substances which, when dissolved in water, resists a pH change in the water, as might be caused by the addition of acid or alkali. Listed below are a few chemicals which, when combined in the proper proportions, will tend to maintain the pH in the indicated range.

Chemicals	pH Range
Acetic Acid + Sodium Acetate	3.7 - 5.6
Sodium Dihydrogen Phosphate + Disodium Hydrogen Phosphate	5.8 - 8.0
Boric Acid + Borax	6.8 - 9.2
Borax + Sodium Hydroxide	9.2 - 11.0

TABLE 3. pH Range of Buffers

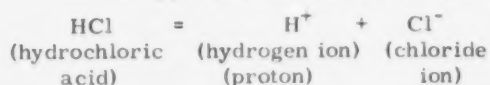
- B A buffer functions by supplying ions which will react with hydrogen ions (acid "spill"), or with hydroxide ions (alkali "spill").
- C In many instances, the buffer is composed of a weak acid and a salt of the weak acid; e.g., acetic acid and sodium acetate.

- 1 In water, acetic acid ionizes or "breaks down" into hydrogen ions and acetate ions.



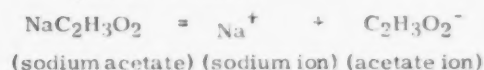
This ionization occurs to only a slight extent, however, most of the acetic acid remains in the form of $\text{HC}_2\text{H}_3\text{O}_2$; only a small amount of hydrogen and acetate ions is formed.

- 2 Thus, acetic acid is said to be a weak acid.
- 3 In the case of other acids, ionization into the component ions occurs to a large degree, and the term strong acid is applied; e.g., hydrochloric acid.



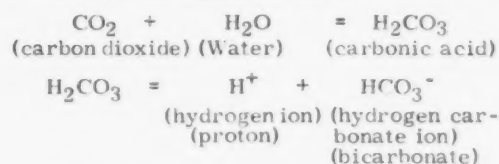
- 4 The terms "strong" and "weak" are also applied to bases. In water solutions, those which break down into their component ions to a large extent are termed "strong", and those which do not are "weak". Sodium hydroxide is a relatively strong base, while ammonium hydroxide is relatively weak.

- 5 Sodium acetate (a salt of acetic acid) dissociates or "breaks down" into sodium ions and acetate ions when placed in water.

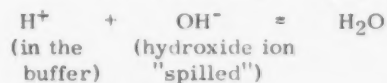


This dissociation occurs to a large extent, and practically all of the sodium acetate is in the form of sodium ions and acetate ions.

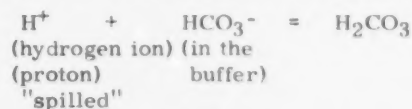
- D It would be difficult and expensive to prepare large quantities of buffers for use in a treatment plant. However, certain naturally occurring buffers may be available (carbon dioxide is an example). It dissolves in water to form the species indicated below.



The hydrogen ions react with hydroxide ions which might appear in the water as the result of an alkali "spill".



The hydrogen carbonate ions react with hydrogen ions which might appear in the water as the result of an acid "spill".



This buffering action will be in effect as long as there is carbonic acid present.

- E Buffering action is not identical with a process in which acid wastes are "neutralized" with alkali wastes, or conversely. The desired effect is achieved in both cases, however (i.e., the pH is maintained within a desired range.)

This outline was prepared by C. R. Feldmann,
Chemist, National Training Center, MDS,
WFO, EPA, Cincinnati, OH 45268

ALKALINITY AND RELATIONSHIPS AMONG THE VARIOUS TYPES OF ALKALINITIES

I PRELIMINARY

The property of water referred to as alkalinity is usually caused by the presence of hydroxyl, carbonate and bicarbonate ions. To a lesser extent, borates, phosphates and silicates contribute but are generally present in negligible amounts.

The concentration and ratio of the OH^- , CO_3^{--} , and HCO_3^- ions may be measured by titrating a sample to certain specified pH's or end points which are detected either by use of a pH meter or by color indicators. Phenolphthalein is used for visual detection of the first end point, (approximately pH 8) which indicates the neutralization of NaOH and conversion of CO_3^{--} to HCO_3^- . A number of indicators (methyl orange, methyl purple, brom cresol green, etc.) are used for detection of the second end point (pH 3-5) which indicates the complete conversion of HCO_3^- to H_2O and CO_2 . The final end point is determined by the amount of CO_3^{--} and HCO_3^- originally present in the sample. If the end points are determined electrometrically they are taken as the mid-point of the greatest rate of pH change per unit volume of titrant.

II RELATIONSHIPS BETWEEN HYDROXIDE, CARBONATE, AND BICARBONATE ALKALINITIES

The results obtained from phenolphthalein and total alkalinity measurements offer a means of classification of the principal forms of alkalinity, if certain assumptions are made. It must first be assumed that interferences are absent and that bicarbonate and hydroxide do not exist in the same solution. According to the system presented in Standard Methods, 13th Edition:

- A Hydroxide alkalinity is present if the phenolphthalein alkalinity is more than one-half the total alkalinity.
- B Carbonate alkalinity is present if the phenolphthalein alkalinity is not zero but is less than the total alkalinity.
- C Bicarbonate alkalinity is present if the phenolphthalein alkalinity is less than one-half the total alkalinity.

Table 1. Relationships Between Phenolphthalein Alkalinity, Total Alkalinity, Carbonate Alkalinity, Bicarbonate Alkalinity and Hydroxide Alkalinity

Lecture Notes	Result of Titration	OH^- Alkalinity as CaCO_3	CO_3^{--} Alkalinity as CaCO_3	HCO_3^- Alkalinity as CaCO_3
Case 1	$P = T$	T	O	O
Case 2	$P = \frac{1}{2}T$	O	2P	O
Case 3	$P = O$	O	O	T
Case 4	$P > \frac{1}{2}T$	2P-T	2(T-P)	O
Case 5	$P < \frac{1}{2}T$	O	2P	T-2P

P = Phenolphthalein Alkalinity

T = Total Alkalinity

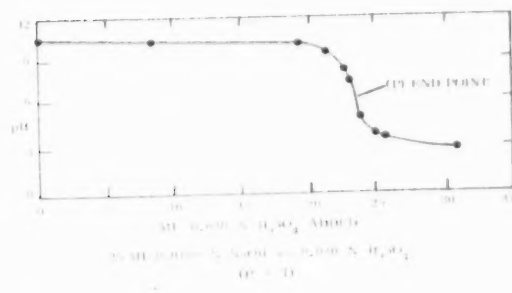
Table 2. Stoichiometric Volumes of Solutions of Different Normalities

Standard Solution	H_2SO_4	NaOH	Na_2CO_3	NaHCO_3
Normality	0.0200	0.0189	0.0199	0.0125
Equivalent Volumes, ml	10.0	10.6	9.9	16.0
	9.4	10.0	9.5	15.1
	10.0	10.5	10.0	15.9
	6.3	6.6	6.3	10.0

III CASE EXAMPLES

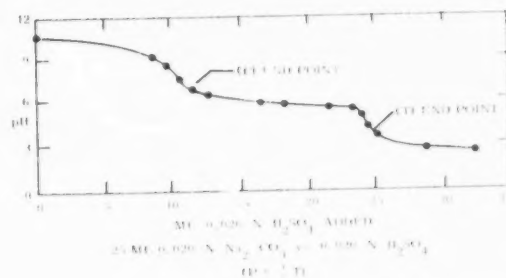
The relationships involved in Table 1 may best be explained by reference to the following graphs. These were prepared by titrating volumes of standard solutions of sodium hydroxide, sodium carbonate, and sodium bicarbonate with standard sulfuric acid. The stoichiometric volumes of the various solutions are summarized in Table 2 for convenience in the interpretation of the charts.

A CASE 1 - Where phenolphthalein alkalinity = total alkalinity



The sharp break occurs at the point where all of the NaOH has been exactly neutralized by the acid. The pH and concentration of the end products (Na_2SO_4 and H_2O) determine the pH at the equivalence point between NaOH and H_2SO_4 ; in this case, approximately 7.0.

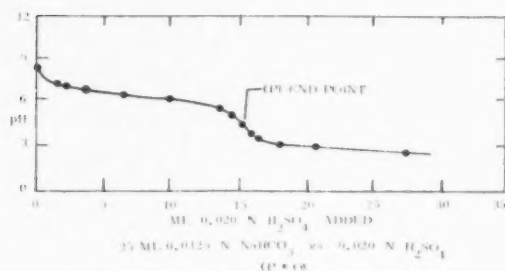
B CASE 2 - Where phenolphthalein alkalinity = one-half the total alkalinity



The titration proceeds in 2 stages wherein all of the CO_3^{--} is converted, first to HCO_3^- and finally to H_2CO_3 . The first end point occurs at approximately pH 8, and at exactly half the volume of acid used for the total titration. The end point which occurs at approximately pH 4 represents the total alkalinity and requires exactly twice the volume of acid used for the first end point.

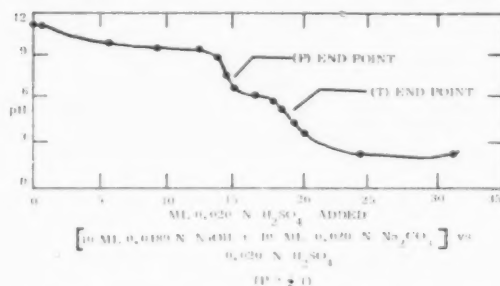
If either HCO_3^- or OH^- ions had been present the titration volumes for the curves would not have been of equal magnitude.

C CASE 3 - Where phenolphthalein alkalinity = 0



T The reaction proceeds in one stage with the initial pH at approximately 8.5 and final pH at 4.0. In this case the phenolphthalein alkalinity is zero and since no conversion of CO_3^{2-} to HCO_3^- is noted the total alkalinity can only be due to the HCO_3^- ion.

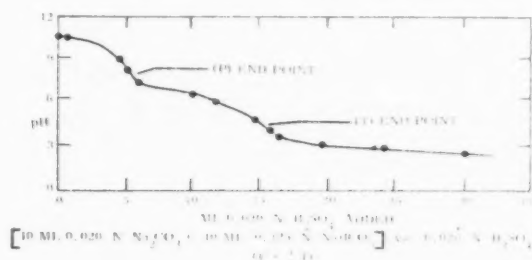
D CASE 4 - Where phenolphthalein alkalinity is greater than one-half the total alkalinity



The volume of acid required for the first end point (phenolphthalein alkalinity) is due to the OH^- neutralization and conversion of the CO_3^{2-} to HCO_3^- . The second end point represents the complete conversion of HCO_3^- to H_2CO_3 . Referring to Case 2 where the volume of acid was

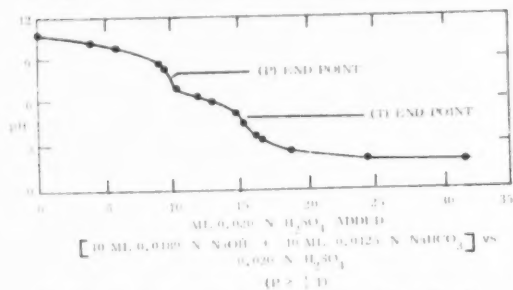
similar for each end point, it is apparent that a base responding to phenolphthalein but which is not CO_3^{2-} must be present. Since it was originally assumed that OH^- and HCO_3^- do not exist in the same solution we must conclude that the total alkalinity is due to OH^- and CO_3^{2-} .

E CASE 5 - Where phenolphthalein alkalinity is less than one half of the total alkalinity



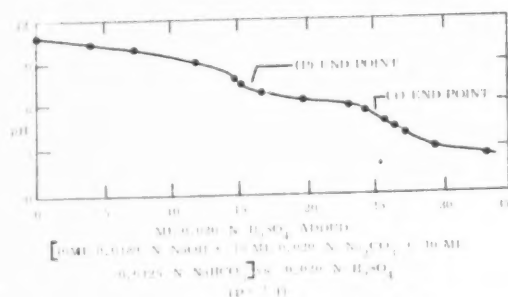
If, in the reaction $NaOH + NaHCO_3 \rightarrow Na_2CO_3 + H_2O$, the $NaOH$ exists in excess quantity, the final sample contains $NaOH$ and Na_2CO_3 , (Case 4) in which the volume of acid required for the phenolphthalein end point is greater than one half the total. In this case, however, the situation is reversed, wherein the volume of acid required for the HCO_3^- end point is greater than one half the total. Referring again to the reaction $NaOH + NaHCO_3 \rightarrow Na_2CO_3 + H_2O$, if $NaHCO_3$ is in excess the end products must consist of Na_2CO_3 and $NaHCO_3$ and OH^- must be absent. The end points consist, therefore, of $Na_2CO_3 \rightarrow NaHCO_3$ (phenolphthalein end point) and $NaHCO_3 \rightarrow H_2CO_3$.

F CASE 6 - Where phenolphthalein alkalinity is greater than one-half total alkalinity.



Following the original assumption that OH^- and HCO_3^- are not compatible, with HCO_3^- being converted to CO_3^{--} we have a condition similar to Case 4 ($P > \frac{1}{2} T$).

G CASE 7 - Where phenolphthalein alkalinity is greater than one-half total alkalinity.



The first end point occurs at the stoichiometric sum of the equivalent volumes as follows:

$$(9.4 - 6.3) + \frac{(10 + 12.6)}{2}$$

where $(9.4 - 6.3)$ = volume of N acid required for excess OH^- after $\text{OH}^- + \text{HCO}_3^-$ reaction,

and $\frac{(10 + 12.6)}{2}$ = volume of acid required for conversion of CO_3^{--} to HCO_3^- . The second end point occurs at $\frac{(10 + 12.6)}{2}$ ml, the volume of HCO_3^- which is converted to H_2CO_3 . This then becomes the same as Case 4.

IV COMPARISON OF ANALYTICAL METHODS FOR ALKALINITY (According to the Analytical Reference Service Report JAWWA Vol. 55, No. 5, 1963)

May 1961

DETERMINATION METHODS

TABLE 2--Statistical Summary (cont.)

Method	Year	No. of Values Reported	Concn. Added mg/l	Concn. Determined mg/l			50% Range mg/l	Standard Deviation mg/l
				Mean	Low	High		
Alkalinity								
Methyl orange	1956	38	19	20.0	11.23	30.0	± 2	5.049
	1958	19	17	19.5	16	24	± 1	2.481
	1961	27	42.5	43.7	38	50	± 1.5	3.250
Electrometric	1956	14	19	19.7	19.0	21.0	± 1	0.911
	1958	53	17	19.4	15	25	± 2	2.740
	1961	88	42.5	44.2	38.9	57	± 2.5	3.605
Methyl purple	1956	8	19	14.7	14.0	15.2	± 4	0.415
	1958	10	17	19.3	18	21	± 2	0.823
	1961	18	42.5	44.8	40	50	± 1.5	3.081
Mixed indicator	1958	4	17	19.5	18	20	± 3	1.000
	1961	15	42.5	42.8	39	49	± 3	3.550
Brom cresol green	1958	1	17	19	19	19	± 2	4.460
	1956	60	19	19.2	11.23	40.0	± 2	2.320
All methods	1958	92	17	19.6	15	25	± 2	2.320
	1961	178	42.5	43.9	25	57	± 1.9	5.135

Alkalinity - The methods for alkalinity measurement varied only in the choice of indicator or pH for determining the end point of the titration. The indicators used included methyl orange, methyl purple, and mixed indicator. The data shows that as the use of electrometric end point increased, the use of methyl orange decreased.

SUMMARY

Amount Added	17 mg/l (as CaCO_3)
Avg. Deviation from amount added	3.1 mg/l
Standard Deviation	3.4 mg/l
50% Range	2.0 mg/l
Method Most Commonly Used	Electrometric
Method Preferred	Electrometric
Total Number of Observations	41

V PROCEDURE

The actual measurement of alkalinity is a very simple procedure requiring only titration of sample with a standardized acid and the proper indicator. For phenolphthalein alkalinity the end point and indicator are well established. For the bicarbonate titration - the final end point is a function of the HCO_3^- concentration. With low amounts ($< 50 \text{ mg/l}$ as CaCO_3) the pH at end point may be approximately 5.0. With high concentrations ($> 250 \text{ mg/l}$ as CaCO_3) the pH at end point may be 4.5 to 3.8. For all-purpose work, in which the highest degree of accuracy is not required, an end point at pH 4.5 using methyl purple as the indicator is recommended.

The traditional methyl orange frequently proves to be unsatisfactory because of the indefinite color change at the end point and also because of the low pH (3.8 - 3.9) required to establish the change.

VI EPA ANALYTICAL METHODS

- A The Environmental Protection Agency, Office of Water Programs, Analytical Quality Control Laboratory has compiled a manual of Analytical Methods which is to be used in Federal laboratories for the chemical analysis of water and waste samples. The title of this manual is "Methods for Chemical Analysis of Water and Wastes, 1971."
- B This manual lists two parameters which are related to the subject matter in this outline. They are total alkalinity and total acidity.
- C For the measurement of both of these parameters, the recommended method is volumetric, with the equivalence point being determined electrometrically. The use of a color indicator (methyl orange) is recommended only for the automated method.
- D The procedure references cited by the EPA Methods manual are the 13th Edition of Standards Methods and ASTM Standards, Part 23, 1970.

REFERENCES

- 1 Standards Methods for the Examination of Water and Wastewater, 12th Ed. APHA, Inc., New York. 1965.
- 2 ASTM Standards, Part 23. 1968.

This outline was prepared by R. C. Kroner, Chief, Physical and Chemical Methods, Analytical Quality Control Laboratory, National Environmental Research Center, Cincinnati, OH 45268.

IV. OXYGEN TESTS

	<u>Outline Number</u>
Dissolved Oxygen Determination (DO)-I (Winkler Iodometric Titration and Azide Modification)	9
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DISSOLVED OXYGEN DETERMINATION (DO) - I

Winkler Iodometric Titration and Azide Modification

I The Dissolved Oxygen determination is a very important water quality criteria for many reasons:

A Oxygen is an essential nutrient for all living organisms. Dissolved oxygen is essential for survival of aerobic organisms and permits facultative organisms to metabolize more effectively. Many desirable varieties of macro or micro organisms cannot survive at dissolved oxygen concentrations below certain minimum values. These values vary with the type of organisms, stage in their life history, activity, and other factors.

B Dissolved oxygen levels may be used as an indicator of pollution by oxygen demanding wastes. Low DO concentrations are likely to be associated with low quality waters.

C The presence of dissolved oxygen prevents or minimizes the onset of putrefactive decomposition and the production of objectionable amounts of malodorous sulfides, mercaptans, amines, etc.

D Dissolved oxygen is essential for terminal stabilization wastewaters. High DO concentrations are normally associated with good quality water.

E Dissolved oxygen changes with respect to time, depth or section of a water mass are useful to indicate the degree of stability or mixing characteristics of that situation.

F The BOD or other respirometric test methods for water quality are commonly based upon the difference among an initial and final DO determination for a given sample time interval and condition. These measurements are useful to indicate:

- 1 The rate of biochemical activity in terms of oxygen demand for a given sample and conditions.
 - 2 The degree of acceptability (a bioassay technique) for biochemical stabilization of a given microbiota in response to food, inhibitory agents or test conditions.
 - 3 The degree of instability of a water mass on the basis of test sample DO changes over an extended interval of time.
 - 4 Permissible load variations in surface water or treatment units in terms of DO depletion versus time, concentration, or ratio of food to organism mass, solids, or volume ratios.
 - 5 Oxygenation requirements necessary to meet the oxygen demand in treatment units or surface water situations.
- G The DO test is the only chemical test included in all Water Quality Criteria, Federal, State, Regional or local.

II FACTORS AFFECTING THE DO CONCENTRATION IN WATER

A Physical Factors:

- 1 DO solubility in water for an air/water system is limited to about 9 mg DO/liter of water at 20°C. This amounts to about 0.0009% as compared to 21% by weight of oxygen in air.
- 2 Transfer of oxygen from air to water is limited by the interface area, the oxygen deficit, partial pressure, the conditions at the

interface area, mixing phenomena and other items.

Certain factors tend to confuse reoxygenation mechanisms of water aeration:

- a The transfer of oxygen in air to dissolved molecular oxygen in water has two principal variables:

- 1) Area of the air-water interface.
- 2) Dispersion of the oxygen-saturated water at the interface into the body liquid.

The first depends upon the surface area of the air bubbles in the water or water drops in the air; the second depends upon the mixing energy in the liquid. If diffusers are placed in a line along the wall, dead spots may develop in the core. Different diffuser placement or mixing energy may improve oxygen transfer to the liquid two or threefold.

- b Other variables in oxygen transfer include:

- 3) Oxygen deficit in the liquid.
- 4) Oxygen content of the gas phase.
- 5) Time.

If the first four variables are favorable, the process of water oxygenation is rapid until the liquid approaches saturation. Much more energy and time are required to increase oxygen saturation from about 95 to 100% than to increase oxygen saturation from 0 to about

95%. For example: An oxygen-depleted sample often will pick up significant DO during DO testing; changes are unlikely with a sample containing equilibrium amounts of DO.

- c The limited solubility of oxygen in water compared to the oxygen content of air does not require the interchange of a large mass of oxygen per unit volume of water to change DO saturation. DO increases from zero to 50% saturation are common in passage over a weir.

- d Aeration of dirty water is practiced for cleanup. Aeration of clean water results in washing the air and transferring fine particulates and gaseous contaminants to the liquid.

- e One liter of air at room temperature contains about 230 mg of oxygen. A 5 gal carboy of water with 2 liters of gas space above the liquid has ample oxygen supply for equilibration of DO after storage for 2 or 3 days or shaking for 30 sec.

- f Aeration tends toward evaporative cooling. Oxygen content becomes higher than saturation values at the test temperature, thus contributing to high blanks.

- 3 Oxygen solubility varies with the temperature of the water. Solubility at 10°C is about two times that at 30°C. Temperature often contributes to DO variations much greater than anticipated by

solubility. A cold water often has much more DO than the solubility limits at laboratory temperature. Standing during warmup commonly results in a loss of DO due to oxygen diffusion from the super-saturated sample. Samples warmer than laboratory temperature may decrease in volume due to the contraction of liquid as temperature is lowered. The full bottle at higher temperature will be partially full after shrinkage with air entrance around the stopper to replace the void. Oxygen in the air may be transferred to raise the sample DO. For example, a volumetric flask filled to the 1000 ml mark at 30°C will show a water level about 1/2 inch below the mark when the water temperature is reduced to 20°C. BOD dilutions should be adjusted to 20°C + or - 1 1/2° before filling and testing.

- 4 Water density varies with temperature with maximum water density at 4°C. Colder or warmer waters tend to promote stratification of water that interferes with distribution of DO because the higher density waters tend to seek the lower levels.
- 5 Oxygen diffusion in a water mass is relatively slow, hence vertical and lateral mixing are essential to maintain relatively uniform oxygen concentrations in a water mass.
- 6 Increasing salt concentration decreases oxygen solubility slightly but has a larger effect upon density stratification in a water mass.
- 7 The partial pressure of the oxygen in the gas above the water interface controls the oxygen solubility limits in the water. For example, the equilibrium concentration of oxygen in water is about 9 mg DO/l under one atmospheric pressure of

air, about 42 mg DO/liter in contact with pure oxygen and 0 mg DO/liter in contact with pure nitrogen (@ 20°C).

B Biological or Bio-Chemical Factors

- 1 Aquatic life requires oxygen for respiration to meet energy requirements for growth, reproduction, and motion. The net effect is to deplete oxygen resources in the water at a rate controlled by the type, activity, and mass of living materials present, the availability of food and favorability of conditions.
- 2 Algae, autotrophic bacteria, plants or other organisms capable of photosynthesis may use light energy to synthesize cell materials from mineralized nutrients with oxygen released in process.
 - a Photosynthesis occurs only under the influence of adequate light intensity.
 - b Respiration of alga is continuous.
 - c The dominant effect in terms of oxygen assets or liabilities of alga depends upon algal activity, numbers and light intensity. Gross algal productivity contributes to significant diurnal DO variations.
- 3 High rate deoxygenation commonly accompanies assimilation of readily available nutrients and conversion into cell mass or storage products. Deoxygenation due to cell mass respiration commonly occurs at some lower rate dependent upon the nature of the organisms present, the stage of decomposition and the degree of predation, lysis, mixing and regrowth. Relatively high

deoxygenation rates commonly are associated with significant growth or regrowth of organisms.

- 4 Micro-organisms tend to flocculate or agglomerate to form settleable masses particularly at limiting nutrient levels (after available nutrients have been assimilated or the number of organisms are large in proportion to available food).
 - a Resulting benthic deposits continue to respire as bed loads.
 - b Oxygen availability is limited because the deposit is physically removed from the source of surface oxygenation and algal activity usually is more favorable near the surface. Stratification is likely to limit oxygen transfer to the bed load vicinity.
 - c The bed load commonly is oxygen deficient and decomposes by anaerobic action.
 - d Anaerobic action commonly is characterized by a dominant hydrolytic or solubilizing action with relatively low rate growth of organisms.
 - e The net effect is to produce low molecular weight products from cell mass with a correspondingly large fraction of feedback of nutrients to the overlaying waters. These lysis products have the effect of a high rate or immediate oxygen demand upon mixture with oxygen containing waters.
 - f Turbulence favoring mixing of surface waters and benthic sediments commonly are associated with extremely rapid depletion of DO.

Recurrent resuspension of thin benthic deposits may contribute to highly erratic DO patterns.

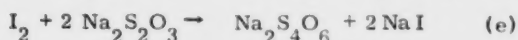
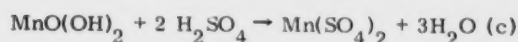
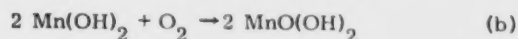
- g Long term deposition areas commonly act like point sources of new pollution as a result of the feedback of nutrients from the deposit. Rate of reaction may be low for old materials but a low percentage of a large mass of unstable material may produce excessive oxygen demands.
- C Tremendous DO variations are likely in a polluted water in reference to depth, cross section or time of day. More stabilized waters tend to show decreased DO variations although it is likely that natural deposits such as leaf mold will produce differences related to depth in stratified deep waters.

III ANALYTICAL METHOD BACKGROUND

The basic Winkler procedure (1888) has been modified many times to improve its workability in polluted waters. None of these modifications have been completely successful. The most useful modification was proposed by Alsterberg and consists of the addition of sodium azide to control nitrite interference during the iodometric titration. The Azide modification of the iodometric titration is recommended as official by the EPA-OWP Quality Control Committee for relatively clean waters.

A Reactions

- 1 The determination of DO involves a complex series of interactions that must be quantitative to provide a valid DO result. The number of sequential reactions also complicates interference control. The reactions will be presented first followed by discussion of the functional aspects.



2 Reaction sequence

The series of reactions involves five different operational steps in converting dissolved oxygen in the water into a form in which it can be measured.

- a $\text{O}_2 \rightarrow \text{MnO(OH)}_2 \rightarrow \text{Mn(SO}_4)_2 \rightarrow \text{I}_2$
Thiosulfate (thio) or phenylarsine oxide (PAO) titration.
 - b All added reagents are in excess to improve contact possibilities and to force the reaction toward completion.
- 3 The first conversion, $\text{O}_2 \rightarrow \text{MnO(OH)}_2$ (reactions a, b) is an oxygen transfer operation where the dissolved oxygen in the water combines with manganous hydroxide to form an oxygenated manganic hydroxide.
 - a The manganous salt can react with oxygen only in a highly alkaline media.
 - b The manganous salt and alkali must be added separately with addition below the surface of the sample to minimize reaction with atmospheric oxygen via air bubbles or surface contact. Reaction with sample dissolved oxygen is intended to occur upon mixing of the reagents and sample after stoppering the full bottle (care should be used to allow entrained air bubbles to rise to the surface before adding reagents to prevent high results due to including entrained oxygen).
 - c Transfer of oxygen from the dissolved state to the precipitate form involves a two phase system of solution and precipitate requiring effective mixing for quantitative transfer. Normally a gross excess of reagents are used to limit mixing requirements. Mixing by rapid inversion 25 to 35 times will accomplish the purpose. Less energy is required by inversion 5 or 6 times, allowing the solids to settle half way and repeat the process. Reaction is rapid; contact is the principal problem in the two phase system.
 - d If the alkaline floc is white, no oxygen is present.
 - 4 Acidification (reactions c and d) changes the oxygenated manganic hydroxide to manganic sulfate which in turn reacts with potassium iodide to form elemental iodine. Under acid conditions, oxygen cannot react directly with the excess manganous sulfate remaining in solution.
 - 5 Iodine (reaction e) may be titrated with sodium thiosulfate standard solution to indicate the amount of dissolved oxygen originally present in the sample.
 - a The blue color of the starch-iodine complex commonly is used as an indicator. This blue color disappears when elemental iodine has been reacted with an equivalent amount of thiosulfate.

- b Phenylarsine oxide solutions **are** more expensive to obtain but have better keeping qualities than thiosulfate solutions. Occasional use, field operations and situations where it is not feasible to calibrate thio solutions regularly, usually encourage use of purchased PAO reagents.

- 6 For practical purposes the DO determination scheme involves the following operations.

- a Fill a 300 ml bottle* under conditions minimizing DO changes. This means that the sample bottle must be flushed with test solution to displace the air in the bottle with water characteristic of the tested sample*.

*DO test bottle volumes should be checked - discard those outside of the limits of 300 ml + or - 3 ml.

- b To the filled bottle:

- 1) Add MnSO_4 reagent (2 ml)
- 2) Add KOH, KI, NaN_3 reagent (2 ml)
Stopper, mix by inversion, allow to settle half way and repeat the operation. Highly saline test waters commonly settle very slowly at this stage and may not settle to the half way point in the time allotted.

- c To the alkaline mix (settled about half way) add 2 ml of sulfuric acid, stopper and mix until the precipitate dissolves.

- d Transfer the contents of the bottle to a 500 ml Erlenmeyer flask and titrate with 0.0375 Normal Thiosulfate*. Each ml of reagent used represents 1 mg of DO/liter of sample.

The same thing applies for other sample volumes when using an appropriate titrant normality such as:

- 1) For a 200 ml sample, use 0.025 N Thio
- 2) For a 100 ml sample, use 0.0125 N Thio

*EPA-WPO Method

- 7 The addition of the first two DO reagents, (MnSO_4 and the KOH, KI and NaN_3 solutions) displaces an equal quantity of the sample. This is not the case when acid is added because the clear liquid above the floc does not contain dissolved oxygen as all of it should be converted to the particulate MnO(OH)_2 . Some error is introduced by this displacement of sample during dosage of the first two reagents. The error upon addition of 2 ml of each reagent to a 300 ml sample is $\frac{4}{300} \times 100$ or 1.33% loss in DO. This may be corrected by an appropriate factor or by adjustment of reagent normality. It is generally considered small in relation to other errors in sampling, manipulation and interference, hence this error may be recognized but not corrected.
- 8 Reagent preparation and procedural details can be found in reference 1.

IV The sequential reactions for the Chemical DO determination provides several situations where significant interference may occur in application on polluted water, such as:

- A Sampling errors may not be strictly designated as interference but have the same effect of changing sample DO. Inadequate flushing of the bottle contents or exposure to air may raise the DO of low oxygen samples or lower the DO of supersaturated samples.

B Entrained air may be trapped in a DO bottle by:

- 1 Rapid filling of vigorously mixed samples without allowing the entrained air to escape before closing the bottle and adding DO reagents.
- 2 Filling a bottle with low temperature water holding more DO than that in equilibrium after the samples warm to working temperature.
- 3 Aeration is likely to cool the sample permitting more DO to be introduced than can be held at the room or incubator temperatures.
- 4 Samples warmer than working incubator temperatures will be only partially full at equilibrium temperatures.

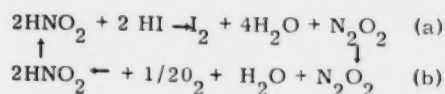
Addition of DO reagents results in reaction with dissolved or entrained oxygen. Results for DO are invalid if there is any evidence of gas bubbles in the sample bottle.

C The DO reagents respond to any oxidant or reductant in the sample capable of reacting within the time allotted. HOCl of H_2O_2 may raise the DO titration while H_2S , 4SH^- may react with sample oxygen to lower the sample titration. The items mentioned react rapidly and raise or lower the DO result promptly. Other items such as Fe^{++} or SO_3 may or may not react completely within the time allotted for reaction. Many organic materials or complexes from benthic deposits may have an effect upon DO results that are difficult to predict. They may have one effect during the alkaline stage to release iodine from KI while favoring irreversible absorption of iodine during the acid stage. Degree of effect may increase with reaction time. It is generally inadvisable to use the iodometric titration on samples containing large amounts of organic contaminants or

benthic residues. It would be expected that benthic residues would tend toward low results because of the reduced iron and sulfur content - they commonly favor high results due to other factors that react more rapidly, often giving the same effect as in uncontrolled nitrite interference during titration.

D Nitrite is present to some extent in natural waters or partially oxidized treatment plant samples. Nitrite is associated with a cyclic reaction during the acid stage of the DO determination that may lead to erroneous high results.

- 1 These reactions may be represented as follows:



These reactions are time, mixing and concentration dependent and can be minimized by rapid processing.

- 2 Sodium azide (NaN_3) reacts with nitrite under acid conditions to form a combination of N_2 + N_2O which effectively blocks the cyclic reaction by converting the HNO_2 to noninterfering compounds of nitrogen.
- 3 Sodium azide added to fresh alkaline KI reagent is adequate to control interference up to about 20 mg of HNO_2 - N/liter of sample. The azide is unstable and gradually decomposes. If resuspended benthic sediments are not detectable in a sample showing a returning blue color, it is likely that the azide has decomposed in the alkaline KI azide reagent.

E Surfactants, color and Fe^{+++} may confuse endpoint detection if present in significant quantities.

- F Polluted water commonly contains significant interferences such as C. It is advisable to use a membrane protected sensor of the electronic type for DO determinations in the presence of these types of interference.
- G The order of reagent addition and prompt completion of the DO determination is critical. Stable waters may give valid DO results after extended delay of titration during the acidified stage. For unstable water, undue delay at any stage of processing accentuates interference problems.

ACKNOWLEDGMENTS

This outline contains significant materials from previous outlines by J. W. Mandia. Review and comments by C. R. Hirth and R. L. Booth are greatly appreciated.

REFERENCE

- 1 Methods for Chemical Analysis of Water and Wastes, EPA-AQCL, Cincinnati, OH, July 1971.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, MDS, WPQ EPA, Cincinnati, OH 45268.

DISSOLVED OXYGEN DETERMINATION - II ELECTRONIC MEASUREMENTS

I INTRODUCTION

A Electronic measurement of DO is attractive for several reasons:

- 1 Electronic methods are more readily adaptable for automated analysis, continuous recording, remote sensing or portability.
- 2 Application of electronic methods with membrane protection of sensors affords a high degree of interference control.
- 3 Versatility of the electronic system permits design for a particular measurement, situation or use.
- 4 Many more determinations per man-hour are possible with a minor expenditure of time for calibration.

B Electronic methods of analysis impose certain restrictions upon the analyst to insure that the response does, in fact, indicate the item sought.

- 1 The ease of reading the indicator tends to produce a false sense of security. Frequent and careful calibrations are essential to establish workability of the apparatus and validity of its response.
- 2 The use of electronic devices requires a greater degree of competence on the part of the analyst. Understanding of the behavior of oxygen must be supplemented by an understanding of the particular instrument and its behavior during use.

C Definitions

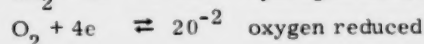
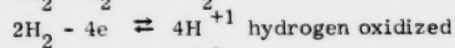
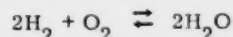
- 1 Electrochemistry - a branch of chemistry dealing with relationships between electrical and chemical changes.

2 Electronic measurements or electro-metric procedures - procedures using the measurement of potential differences as an indicator of reactions taking place at an electrode or plate.

3 Reduction - any process in which one or more electrons are added to an atom or an ion, such as $O_2 + 2e \rightarrow 2O^-$. The oxygen has been reduced.

4 Oxidation - any process in which one or more electrons are removed from an atom or an ion, such as $Zn^0 - 2e \rightarrow Zn^{+2}$. The zinc has been oxidized.

5 Oxidation - reduction reactions - in a strictly chemical reaction, reduction cannot occur unless an equivalent amount of some oxidizable substance has been oxidized. For example:



Chemical reduction of oxygen may also be accomplished by electrons supplied to a noble metal electrode by a battery or other energizer.

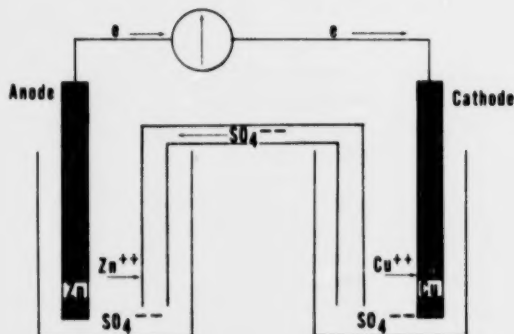
6 Anode - an electrode at which oxidation of some reactable substance occurs.

7 Cathode - an electrode at which reduction of some reactable substance occurs. For example in I.C. 3, the reduction of oxygen occurs at the cathode.

8 Electrochemical reaction - a reaction involving simultaneous conversion of chemical energy into electrical energy or the reverse. These conversions are

equivalent in terms of chemical and electrical energy and generally are reversible.

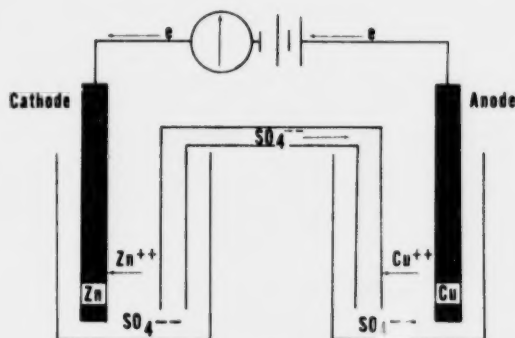
- 9 Electrolyte a solution, gel, or mixture capable of conducting electrical energy and serving as a reacting media for chemical changes. The electrolyte commonly contains an appropriate concentration of selected mobile ions to promote the desired reactions.
- 10 Electrochemical cell - a device consisting of an electrolyte in which 2 electrodes are immersed and connected via an external metallic conductor. The electrodes may be in separate compartments connected by tube containing electrolyte to complete the internal circuit.
 - a Galvanic (or voltaic) cell - an electrochemical cell operated in such a way as to produce electrical energy from a chemical change, such as a battery (See Figure 1).



GALVANIC CELL

Figure 1

- b Polarographic (electrolytic) cell - an electrochemical cell operated in such a way as to produce a chemical change from electrical energy (See Figure 2).



POLAROGRAPHIC CELL

Figure 2

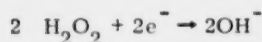
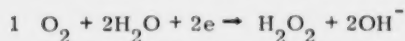
- D As indicated in I. C. 10 the sign of an electrode may change as a result of the operating mode. The conversion by the reactant of primary interest at a given electrode therefore designates terminology for that electrode and operating mode. In electronic oxygen analyzers, the electrode at which oxygen reduction occurs is designated the cathode.
- E Each cell type has characteristic advantages and limitations. Both may be used effectively.
 - 1 The galvanic cell depends upon measurement of electrical energy produced as a result of oxygen

reduction. If the oxygen content of the sample is negligible, the measured current is very low and indicator driving force is negligible, therefore response time is longer.

- 2 The polarographic cell uses a standing current to provide energy for oxygen reduction. The indicator response depends upon a change in the standing current as a result of electrons released during oxygen reduction. Indicator response time therefore is not dependent upon oxygen concentration.
- 3 Choice may depend upon availability, habit, accessories, or the situation. In each case it is necessary to use care and judgment both in selection and use for the objectives desired.

II ELECTRONIC MEASUREMENT OF DO

- A Reduction of oxygen takes place in two steps as shown in the following equations:



Both equations require electron input to activate reduction of oxygen. The first reaction is more important for electronic DO measurement because it occurs at a potential (voltage) which is below that required to activate reduction of most interfering components (0.3 to 0.8 volts relative to the saturated calomel electrode - SCE). Interferences that may be reduced at or below that required for oxygen usually are present at lower concentrations in water or may be minimized by the use of a selective membrane or other means. When reduction occurs, a definite quantity of electrical energy is produced that is proportional to the quantity of reductant entering the reaction. Resulting current measurements thus are more specific for oxygen reduction.

- B Most electronic measurements of oxygen are based upon one of two techniques for evaluating oxygen reduction in line with

equation II.A.1. Both require activating energy, both produce a current proportional to the quantity of reacting reductant. The techniques differ in the means of supplying the activating potential; one employs a source of outside energy, the other uses spontaneous energy produced by the electrode pair.

- 1 The polarographic oxygen sensor relies upon an outside source of potential to activate oxygen reduction. Electron gain by oxygen changes the reference voltage.
 - a Traditionally, the dropping mercury electrode (DME) has been used for polarographic measurements. Good results have been obtained for DO using the DME but the difficulty of maintaining a constant mercury drop rate, temperature control, and freedom from turbulence makes it impractical for field use.
 - b Solid electrodes are attractive because greater surface area improves sensitivity. Poisoning of the solid surface electrodes is a recurrent problem. The use of selective membranes over noble metal electrodes has minimized but not eliminated electrode contamination. Feasibility has been improved sufficiently to make this type popular for regular use.
- 2 Galvanic oxygen electrodes consist of a decomposable anode and a noble metal cathode in a suitable electrolyte to produce activating energy for oxygen reduction (an air cell or battery). Lead is commonly used as the anode because its decomposition potential favors spontaneous reduction of oxygen. The process is continuous as long as lead and oxygen are in contact in the electrolyte and the electrical energy released at the cathode may be dissipated by an outside circuit. The anode may be conserved by limiting oxygen availability. Interrupting the outside circuit may produce erratic behavior for a time after reconnection. The resulting

current produced by oxygen reduction may be converted to oxygen concentration by use of a sensitivity coefficient obtained during calibration. Provision of a pulsed or interrupted signal makes it possible to amplify or control the signal and adjust it for direct reading in terms of oxygen concentration or to compensate for temperature effects.

III ELECTRONIC DO ANALYZER APPLICATION FACTORS

A Polarographic or galvanic DO instruments operate as a result of oxygen partial pressure at the sensor surface to produce a signal characteristic of oxygen reduced at the cathode of some electrode pair. This signal is conveyed to an indicating device with or without modification for sensitivity and temperature or other influences depending upon the instrument capabilities and intended use.

- 1 Many approaches and refinements have been used to improve workability, applicability, validity, stability and control of variables. Developments are continuing. It is possible to produce a device capable of meeting any reasonable situation, but situations differ.
- 2 Most commercial DO instruments are designed for use under specified conditions. Some are more versatile than others. Benefits are commonly reflected in the price. It is essential to determine the requirements of the measurement situation and objectives for use. Evaluation of a given instrument in terms of sensitivity, response time, portability, stability, service characteristics, degree of automation, and consistency are used for judgment on a cost/benefit basis to select the most acceptable unit.

B Variables Affecting Electronic DO Measurement

- 1 Temperature affects the solubility of oxygen, the magnitude of the resulting signal and the permeability of the

protective membrane. A curve of oxygen solubility in water versus increasing temperature may be concave downward while a similar curve of sensor response versus temperature is concave upward. Increasing temperature decreases oxygen solubility and increases probe sensitivity and membrane permeability. Thermistor actuated compensation of probe response based upon a linear relationship or average of oxygen solubility and electrode sensitivity is not precisely correct as the maximum spread in curvature occurs at about 17°C with lower deviations from linearity above or below that temperature. If the instrument is calibrated at a temperature within + or - 5°C of working temperature, the compensated readout is likely to be within 2% of the real value. Depending upon probe geometry, the laboratory sensor may require 4 to 6% correction of signal per °C change in liquid temperature.

- 2 Increasing pressure tends to increase electrode response by compression and contact effects upon the electrolyte, dissolved gases and electrode surfaces. As long as entrained gases are not contained in the electrolyte or under the membrane, these effects are negligible.

Inclusion of entrained gases results in erratic response that increases with depth of immersion.

- 3 Electrode sensitivity changes occur as a result of the nature and concentration of contaminants at the electrode surfaces and possible physical chemical or electronic side reactions produced. These may take the form of a physical barrier, internal short, high residual current, or chemical changes in the metal surface. The membrane is intended to allow dissolved gas penetration but to exclude passage of ions or particulates. Apparently some ions or materials producing extraneous ions within the electrode vicinity are able to pass in limited amounts which

become significant in time. Dissolved gases include 1) oxygen, 2) nitrogen, 3) carbon dioxide, 4) hydrogen sulfide, and certain others. Item 4 is likely to be a major problem. Item 3 may produce deposits in alkaline media; most electrolytes are alkaline or tend to become so in line with reaction II.A.1. The usable life of the sensor varies with the type of electrode system, surface area, amount of electrolyte and type, membrane characteristics, nature of the samples to which the system is exposed and the length of exposure. For example, galvanic electrodes used in activated sludge units showed that the time between cleanup was 4 to 6 months for electrodes used for intermittent daily checks of effluent DO; continuous use in the mixed liquor required electrode cleanup in 2 to 4 weeks. Each electrometric cell configuration and operating mode has its own response characteristics. Some are more stable than others. It is necessary to check calibration frequency required under conditions of use as none of them will maintain uniform response indefinitely. Calibration before and after daily use is advisable.

- 4 Electrolytes may consist of solutions or gels of ionizable materials such as acids, alkalies or salts. Bicarbonates, KCl and KI are frequently used. The electrolyte is the transfer and reaction media, hence, it necessarily becomes contaminated before damage to the electrode surface may occur. Electrolyte concentration, nature, amount and quality affect response time, sensitivity, stability, and specificity of the sensor system. Generally a small quantity of electrolyte gives a shorter response time and higher sensitivity but also may be affected to a greater extent by a given quantity of contaminating substances.
- 5 Membranes may consist of teflon, polyethylene, rubber, and certain other polymeric films. Thickness may vary from 0.5 to 3 mils (inches $\times$ 1/1000). A thinner membrane will

decrease response time and increase sensitivity but is less selective and may be ruptured more easily. The choice of material and its uniformity affects response time, selectivity and durability. The area of the membrane and its permeability are directly related to the quantity of transported materials that may produce a signal. The permeability of the membrane material is related to temperature and to residues accumulated on the membrane surface or interior. A cloudy membrane usually indicates deposition and more or less loss of signal.

- 6 Test media characteristics control the interval of usable life between cleaning and rejuvenation for any type of electrode. More frequent cleanup is essential in low quality waters than for high quality waters. Reduced sulfur compounds are among the more troublesome contaminants. Salinity affects the partial pressure of oxygen at any given temperature. This effect is small compared to most other variables but is significant if salinity changes by more than 500 mg/l.
- 7 Agitation of the sample in the vicinity of the electrode is important because DO is reduced at the cathode. Under quiescent conditions a gradient in dissolved oxygen content would be established on the sample side of the membrane as well as on the electrode side, resulting in atypical response. The sample should be agitated sufficiently to deliver a representative portion of the main body of the liquid to the outer face of the membrane. It is commonly observed that no agitation will result in a very low or negligible response after a short period of time. Increasing agitation will cause the response to rise gradually until some minimum liquid velocity is reached that will not cause a further increase in response with increased mixing energy. It is important to check mixing velocity to reach a stable high signal that is independent of a reasonable change in sample mixing. Excessive

mixing may create a vortex and expose the sensing surface to air rather than sample liquid. This should be avoided. A linear liquid velocity of about 1 ft/sec at the sensing surface is usually adequate.

- 8 DO sensor response represents a potential or current signal in the milli-volt or milli-amp range in a high resistance system. A high quality electronic instrument is essential to maintain a usable signal-to-noise ratio. Some of the more common difficulties include:

- a Variable line voltage or low batteries in amplifier power circuits.
- b Substandard or unsteady amplifier or resistor components.
- c Undependable contacts or junctions in the sensor, connecting cables, or instrument control circuits.
- d Inadequately shielded electronic components.
- e Excessive exposure to moisture, fumes or chemicals in the wrong places lead to stray currents, internal shorts or other malfunction.

C Desirable Features in a Portable DO Analyzer

- 1 The unit should include steady state performance electronic and indicating components in a convenient but sturdy package that is small enough to carry.
- 2 There should be provisions for addition of special accessories such as bottle or field sensors, agitators, recorders, line extensions, if needed for specific requirements. Such additions should be readily attachable and detachable and maintain good working characteristics.
- 3 The instrument should include a sensitivity adjustment which upon calibration will provide for direct reading in terms of mg of DO/liter.

- 4 Temperature compensation and temperature readout should be incorporated.

- 5 Plug in contacts should be positive, sturdy, readily cleanable and situated to minimize contamination. Water seals should be provided where necessary.

- 6 The sensor should be suitably designed for the purpose intended in terms of sensitivity, response, stability, and protection during use. It should be easy to clean, and reassemble for use with a minimum loss of service time.

- 7 Switches, connecting plugs, and contacts preferably should be located on or in the instrument box rather than at the "wet" end of the line near the sensor. Connecting cables should be multiple strand to minimize separate lines. Calibration controls should be convenient but designed so that it is not likely that they will be inadvertently shifted during use.

- 8 Agitator accessories for bottle use impose special problems because they should be small, self contained, and readily detachable but sturdy enough to give positive agitation and electrical continuity in a wet zone.

- 9 Major load batteries should be rechargeable or readily replaceable. Line operation should be feasible wherever possible.

- 10 Service and replacement parts availability are a primary consideration. Drawings, parts identification and trouble shooting memos should be incorporated with applicable operating instructions in the instrument manual in an informative organized form.

D Sensor and Instrument Calibration

The instrument box is likely to have some form of check to verify electronics, battery or other power supply conditions for use. The sensor commonly is not included in this check. A known reference

sample used with the instrument in an operating mode is the best available method to compensate for sensor variables under use conditions. It is advisable to calibrate before and after daily use under test conditions. Severe conditions, changes in conditions, or possible damage call for calibrations during the use period. The readout scale is likely to be labeled - calibration is the basis for this label.

The following procedure is recommended:

- 1 Turn the instrument on and allow it to reach a stable condition. Perform the recommended instrument check as outlined in the operating manual.
- 2 The instrument check usually includes an electronic zero correction. Check each instrument against the readout scale with the sensor immersed in an agitated solution of sodium sulfite containing sufficient cobalt chloride to catalyze the reaction of sulfite and oxygen. The indicator should stabilize on the zero reading. If it does not, it may be the result of residual or stray currents, internal shorting in the electrode, or membrane rupture. Minor adjustments may be made using the indicator rather than the electronic controls. Serious imbalance requires electrode reconditioning if the electronic check is O.K. Sulfite must be carefully rinsed from the sensor until the readout stabilizes to prevent carry over to the next sample.
- 3 Fill two DO bottles with replicate samples of clarified water similar to that to be tested. This water should not contain significant test interferences.
- 4 Determine the DO in one by the azide modification of the iodometric titration.
- 5 Insert a magnetic stirrer in the other bottle or use a probe agitator. Start agitation after insertion of the sensor assembly and note the point of stabilization.

a Adjust the instrument calibration control if necessary to compare with the titrated DO.

b If sensitivity adjustment is not possible, note the instrument stabilization point and designate it as u_a . A sensitivity coefficient,

ϕ is equal to $\frac{u_a}{DO}$ where DO is the

titrated value for the sample on which u_a was obtained. An unknown

DO then becomes $DO = \frac{u_a}{\phi}$. This

factor is applicable as long as the sensitivity does not change.

- 6 Objectives of the test program and the type of instrument influence calibration requirements. Precise work may require calibration at 3 points in the DO range of interest instead of at zero and high range DO. One calibration point frequently may be adequate.

Calibration of a DO sensor in air is a quick test for possible changes in sensor response. The difference in oxygen content of air and of water is too large for air calibration to be satisfactory for precise calibration for use in water.

IV This section reviews characteristics of several sample laboratory instruments. Mention of a specific instrument does not imply EPA endorsement or recommendation. No attempt has been made to include all the available instruments; those described are used to indicate the approach used at one stage of development which may or may not represent the current available model.

- A The electrode described by Carrit and Kanwisher (1) is illustrated in Figure 3. This electrode was an early example of those using a membrane. The anode was a silver - silver oxide reference cell with a platinum disc cathode (1-3 cm diameter). The salt bridge consisted of N/2 KCl and

KOH. The polyethylene membrane was held in place by a retaining ring. An applied current was used in a polarographic mode. Temperature effects were relatively large. Thermistor correction was studied but not integrated with early models.

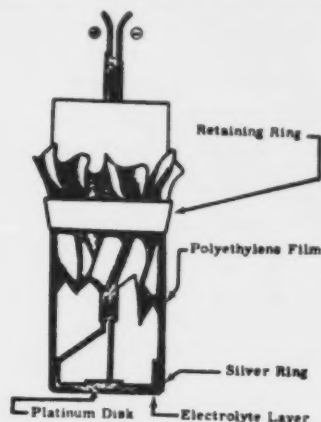


Figure 3

B The Beckman oxygen electrode is another illustration of a polarographic DO sensor (Figure 4). It consists of a gold cathode, a silver anode, an electrolytic gel containing KCl, covered by a teflon membrane. The instrument has a temperature readout and compensating thermistor, a source polarizing current, amplifier with signal adjustment and a readout DO scale with recorder contacts.

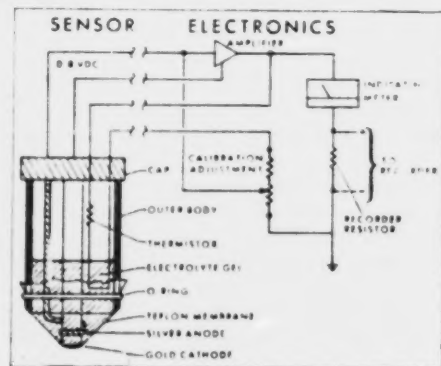
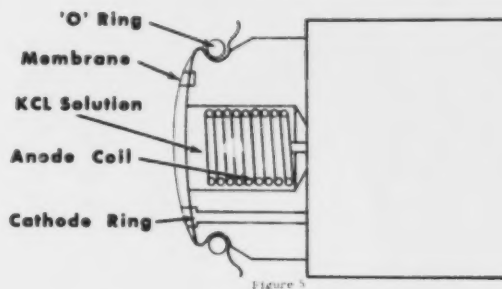


Figure 4. THE BECKMAN OXYGEN SENSOR

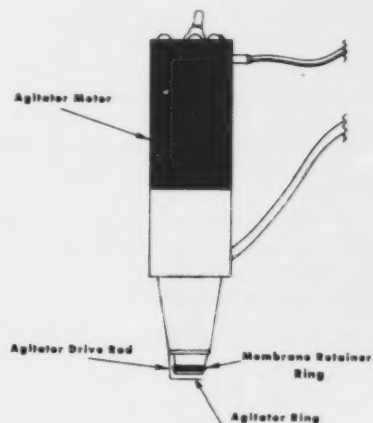
C The YSI Model 51 (3) is illustrated in Figure 5. This is another form of polarographic DO analyzer. The cell consists of a silver anode coil, a gold ring cathode and a KCl electrolyte with a teflon membrane. The instrument has a sensitivity adjustment, temperature and DO readout. The model 51 A has temperature compensation via manual preset dial. A field probe and bottle probe are available.

YSI Model 51 DO Sensor



D The Model 54 YSI DO analyzer (4) is based upon the same electrode configuration but modified to include automatic temperature compensation, DO readout, and recorder jacks. A motorized agitator bottle probe is available for the Model 54 (Figure 6).

YSI Model 54 Agitator Probe



- E The Galvanic Cell Oxygen Analyzer (7, 8) employs an indicator for proportional DO signal but does not include thermistor compensation or signal adjustment. Temperature readout is provided. The sensor includes a lead anode ring, and a silver cathode with KOH electrolyte (4 molar) covered by a membrane film (Figure 7).

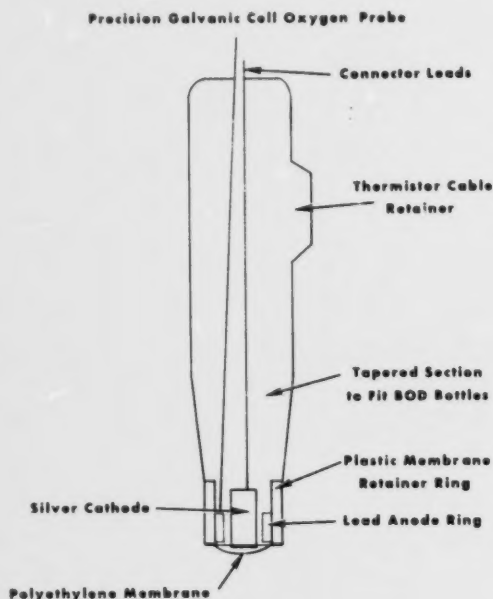


Figure 7

- F The Weston and Stack Model 300 DO Analyzer (8) has a galvanic type sensor with a pulsed current amplifier adjustment to provide for signal and temperature compensation. DO and temperature readout is provided. The main power supply is a rechargeable battery. The sensor (Figure 8) consists of a lead anode coil recessed in the electrolyte cavity (50% KI) with a platinum cathode in the tip. The sensor is covered with a teflon membrane. Membrane retention by rubber band or by a plastic retention ring may be used for the bottle agitator or depth sampler respectively. The thermistor and agitator are mounted in a sleeve that also provides protection for the membrane.

- G The EIL Model 15 A sensor is illustrated in Figure 9. This is a galvanic cell with thermistor activated temperature compensation and readout. Signal adjustment is provided. The illustration shows an expanded scheme of the electrode which when assembled compresses into a sensor approximating 5/8 inch diameter and 4 inch length exclusive of the enlargement at the upper end. The anode consists of compressed lead shot in a replaceable capsule (later models used fine lead wire coils), a perforated silver cathode sleeve around the lead is covered by a membrane film. The electrolyte is saturated potassium bicarbonate. The large area of lead surface, silver and membrane provides a current response of 200 to 300 microamperes in oxygen saturated water at 20°C for periods of up to 100 days use (8). The larger electrode displacement favors a scheme described by Eden (9) for successive DO readings for BOD purposes.

- V Table 1 summarizes major characteristics of the sample DO analyzers described in Section IV. It must be noted that an ingenious analyst may adapt any one of these for special purposes on a do-it-yourself program. The sample instruments are mainly designed for laboratory or portable field use. Those designed for field monitoring purposes may include similar designs or alternate designs generally employing larger anode, cathode, and electrolyte capacity to approach better response stability with some sacrifice in response time and sensitivity. The electronic controls, recording, telemetering, and accessory apparatus generally are semi-permanent installations of a complex nature.

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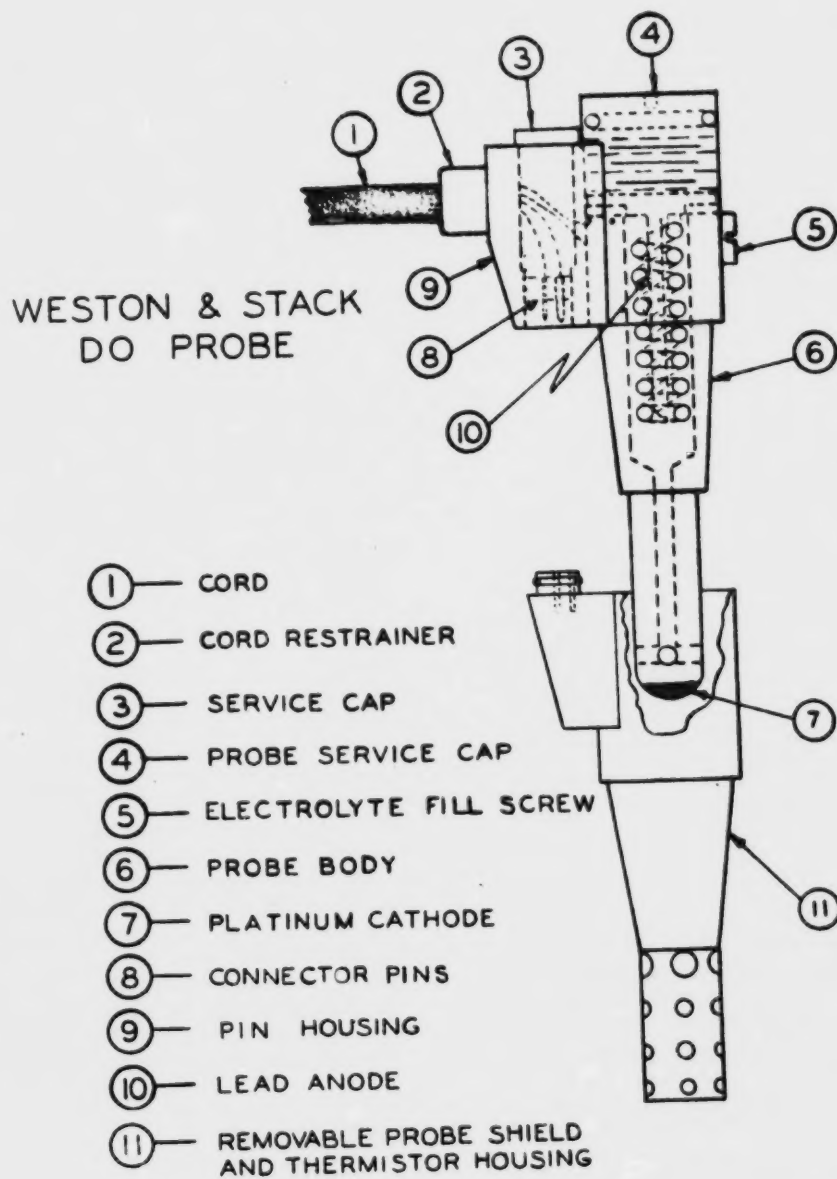
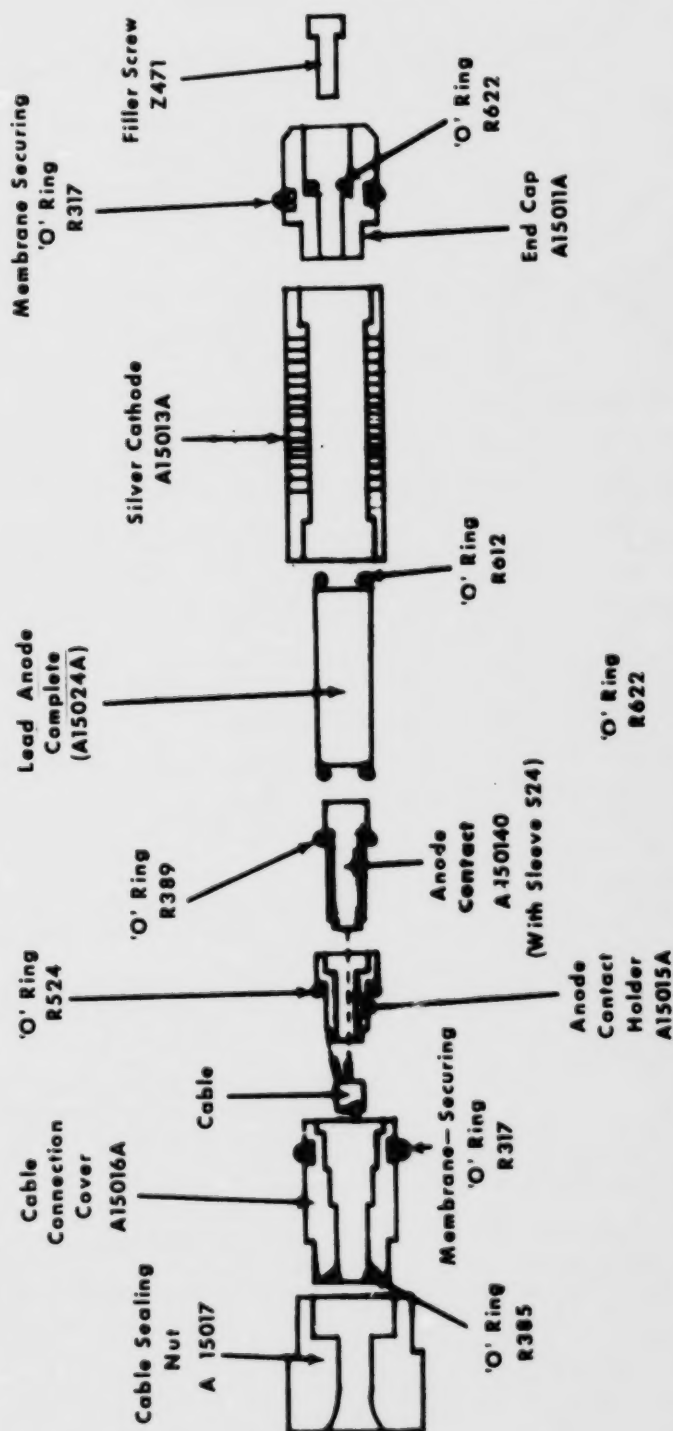


Figure 8

Model A15A ELECTRODE COMPONENT PARTS

Note: Red wire of cable connects to Anode Contact Holder

Black wire of cable connects to Anode Contact

Membrane not shown E. I. L. part number 122

Figure 9

TABLE 1
CHARACTERISTICS OF VARIOUS LABORATORY DO INSTRUMENTS

	Anode	Cathode	Elec	Type	Membr	DO Sig. Adj.	Temp. Comp. Temp.	Accessories for which designed
Carrit & Kanwisher	silver- silver ox. ring	Pt disc	KCl KOH N/2	pol*	polyeth	no	no	Recording temp. & signal adj. self assembled
Beckman	Aq ring	Au disc	KCL gel	pol	teflon	yes	yes yes	recording
Yellow Springs 51	Ag coil	Au ring	KCl soln sat.	pol	teflon	yes	no* yes	field and bottle probe
Yellow Springs 54	"	"	"	"	"	yes	yes yes	recording field bottle & agitator probes
Precision Sci	Pb ring	silver disc	KOH 4N	galv**	polyeth	no	no yes	
Weston & Stack 300	Pb coil	Pt disc	KI 40%	galv	teflon	yes	yes yes	agit. probe depth sampler
EIL	Pb	Ag	KHCO ₃	galv	teflon	yes	yes yes	recording
Delta 75	Lead	Silver disc	KOH 1N	galv	teflon	yes	yes no	field bottle & agitator probe
Delta 85	Lead	Silver disc	KOH 1N	galv	teflon	yes	yes yes	field bottle & agitator probe

*Pol - Polarographic (or amperometric)

**Galv - Galvanic (or voltametric)

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This outline was prepared by F. J. Ludzack, Chemist, National Training Center, DTTB, MDS, Environmental Protection Agency, WPO, Cincinnati, OH 45268 and Nate Malof, Chemist National Field Investigations Center, Environmental Protection Agency, WPO, Cincinnati, OH.

BIOCHEMICAL OXYGEN DEMAND TEST PROCEDURES

I OXYGEN DEMAND OF POLLUTED WATERS

Established practice includes common use of the BOD test as a tool for estimation of the bio-oxidizable fraction of surface waters or wastewaters discharged to them. Any index including a quantity per unit time such as the BOD₅ is a rate expression. The ultimate demand is more important than any one point on the progression. The results of a bottle test with minimum seeding and quiescent storage are not likely to be as high as those on the same influent in a mixing situation and abundant seed of secondary treatment or receiving waters. The BOD₅ is "a" fraction of total oxygen requirements.

- A The particular technique used for BOD commonly is specified by State agencies and/or supervisors. They are required to interpret the results as obtained by laboratory testing. It is essential that the tester and the interpreters have a common understanding of what was done and how. It is highly advisable to maintain a given routine until all concerned agree upon a change.
- 1 Each particular routine has many undefinable factors. The particular routine is not as important as the consistency and capability with which the result was obtained.
 - 2 This outline and Standard Methods⁽¹⁾ discusses several valid approaches for obtaining BOD results. Selection of "method" is not intended in this outline or in the EPA Methods Manual⁽²⁾.
- B The common 5-day incubation period for BOD testing is a result of tradition and cost. Initial lags are likely to be over and some unknown fraction of the total oxidizable mass has been satisfied after 5 days.
- C A series of observations over a period of time makes it possible to estimate the total oxidizable mass and the fraction oxidized or remaining to be oxidized at any given time. The problem is to define

the shape of the deoxygenation pattern and its limits. A fair estimate of the shape of the deoxygenation pattern is available by observations at 1, 2, or 3 days, 7 days and 14 days. Increased observations are desirable for more valid estimates of curve shape, rate of oxidation and total oxidizable mass or ultimate BOD.

- D Increasing impoundment of surface waters and concurrent increases in complexity and stability of wastewater components emphasize the importance of long-term observation of BOD. The 5-day observation includes most of the readily oxidizable materials but a very small fraction of the stable components that are the main factors in impoundment behavior.

II DIRECT METHOD

- A With relatively clean surface waters, the BOD may be determined by incubation of the undiluted sample for the prescribed time interval. This method is applicable only to those waters whose BOD is less than 8 mg/l and assumes the sample contains suitable organisms and accessory nutrients for optimum biological stabilization.

- B Treated effluents, polluted surface waters, household and industrial wastewaters commonly require dilution to provide the excess oxygen required for the oxygen demand determination. General guidelines for dilution requirements for a given BOD range in terms of the percent of sample in BOD dilution water are:

For a 5-day BOD of
5-20 mg/l, use 25 to 100% sample

For a BOD of
20-100 mg/l, use 5 to 25% sample

For a BOD of
100-500 mg/l, use 1 to 5% sample

For a BOD of
500-5000 mg/l, use 0.1 to 1.0% sample

III PROCEDURES

A Cylinder Dilution Technique

- 1 Using an assumed or estimated BOD value as a guide, calculate the factors for a range of dilutions to cover the desired depletions. Those dilutions ranging from a depletion of 2 mg/l and a residual of 1 mg/l are most reliable. At least three dilutions in duplicate should be used for an unknown sample.
- 2 Into a one-liter graduate cylinder (or larger container if necessary) measure accurately the required amount of mixed sample to give one liter of diluted waste. Fill to the one liter mark with dilution water. Carefully mix. The initial DO by calculation includes IDOD (VIII) a determined initial does not. Both are essential to estimate significance of IDOD. Entrapment of air bubbles during manipulation must be avoided.
- 3 Siphon the mixture from the cylinder into three 300 ml glass stoppered bottles, filling the bottles to overflowing.
- 4 Determine the DO concentration on one of the bottles by the appropriate Winkler modification and record as "Initial DO".
- 5 Incubate the two remaining bottles at 20°C in complete darkness. The incubated bottles should be water-sealed by immersion in a tray or by using a special water-seal bottle.
- 6 After 5 days of incubation, or other desired interval, determine the DO on the bottles. Average the DO concentration of the duplicates and report as "Final DO".

B Direct Dilution Technique

- 1 It may be more convenient to make the dilution directly in sample bottles of known capacity. A measured volume of sample may be added (as indicated in A-1) and the bottle filled with dilution water to make the desired sample concentration for incubation. In this case, the sample must be precisely measured, the bottle carefully filled, but not overfilled, and the bottle volumes comparable and known. Precision is likely to be poorer than for cylinder dilution.
- 2 Continue the procedure as in A-4, 5, and 6.

C Seeded Cylinder Dilution Technique

- 1 Many wastewaters may be partially or completely sterile as a result of chlorination, effects of other toxic chemicals, heat, unfavorable pH or other factors detrimental to biological activity. Validity of the BOD result depends upon the presence of organisms capable of prompt and effective biodegradation and favorable conditions during the particular test. Correction of the cause resulting in sterilization must be corrected by adjustment, dilution, etc., prior to reinoculation to achieve meaningful BOD data. Receiving water, biologically treated effluents, and soil suspensions are a good source of organisms likely to be adapted for stabilization of wastewaters. Untreated wastewaters provide numerous organisms but are likely to contain nutrients contributing to excessive seed corrections and may require appreciable time for adaptation before test waste oxidation becomes significant.
- 2 The amount of added inoculant must be determined by trial. The concentration added should initiate biochemical activity promptly but should not exert enough oxygen demand to unduly reduce the oxygen available for sample requirements.
- 3 Estimate the sample concentration desired in accordance with A-1 and C-2 and add the sample aliquot to the dilution cylinder.
- 4 Add approximately half of the required amount of dilution water to the sample and mix. This is necessary to assure that the concentrated waste does not exert a toxic effect on the seed organisms.
- 5 Measure a suitable aliquot of seed into the bottle or cylinder and fill with dilution water. Mix the combined sample, seed and dilution water without excessive air entrainment.
- 6 Continue as in III-A steps 4, 5, and 6.

IV INTERPRETATION OF RESULTS

Standard Methods⁽¹⁾ includes a calculation section that is valid and concise. Preceding it are details of reagent preparation and

procedures for the test. These will not be reprinted here. This section considers certain items that may cause concern about the validity of results unless they are carefully considered and controlled.

A The initial DO of the BOD test obviously should be high. The method of attaining a high DO can trap the analyst.

1 Aeration of dilution water is the most commonly considered treatment. This technique does produce a high DO but it is a treacherous ally.

a Dirty air passing through clean dilution water can produce clean air and dirty water. This is a simple air-washing operation. Filtering the entering air stream may remove brickbats and 2 x 4's, but filters tend to pass organic gases, fine aerosols, and particulates.

b A stream of air passing through water tends to cool the water by evaporation 1 to 3°C below ambient temperature. The cooled liquid picks up more DO than it can hold at ambient temperature. The physical loss of oxygen may produce an erroneously high depletion value for a determined initial DO, or a low depletion on a calculated initial DO. Erroneous blanks are a particular concern. The dilution water temperature/DO shift is critical.

2 Raising DO by allowing the sample to equilibrate in a cotton-plugged bottle for 2 or 3 days permits oxygenation with minimum air volume contact.

3 Shaking a partially filled bottle for a few seconds also oxygenates with minimum opportunity of gas washing contamination, supersaturation, or temperature changes.

B Seeding always is a precarious procedure but a very necessary one at times. Often the application of seed corrections is a

"_____ if you do, _____ if you don't" situation. Hopefully, seed corrections are small because each individual biological situation is a "universe" of its own.

1 Unstable seeding materials such as fresh wastewater have "seed" organisms characteristic of their origin and history. Saprophytes resulting in surface water stabilization may be a small fraction of the population. Reactable oxygen-demanding components produce excessive demands upon test oxygen resources.

2 A seed containing viable organisms at a lower energy state because of limited nutritional availability theoretically is the best available seed source. An organism population grown under similar conditions should be most effective for initiating biochemical activity as soon as the nutrient situation favors more activity. The population should not be stored too long because organism redistribution and die-out become limiting. This type of seed would most likely be found in a surface water or a treatment plant effluent with a history of receiving the particular material under consideration.

3 Seed sources and amounts can only be evaluated by trial. Different seed sources and locations require checkout to determine the best available material from a standpoint of rapid initiation of activity, low correction, and predictable high oxygen depletion under test.

C Chlorination and BOD results fundamentally are incompatible. Chlorination objectives include disinfection as the number one goal. Chlorine is notoriously non-specific in organism effects. Chlorine acts like an oxidant in the DO determination. Test organisms are less suitable for activity than they were before chlorination. Nutrients may be less available after chlorination. Certainly the conditions are less suitable for biological response after chlorination. Dechlorination is feasible with respect to the oxidizing power of

chlorine, but many organic chlorine compounds that do not show strong oxidizing action still have toxic effects on biologic response.

Numbers are obtainable after dechlorination and reseeded. The meaning of these numbers is obscure. At least two states (New York and New Jersey) specify BOD's before chlorination only.

V PRECISION OF THE BOD TEST

- A The DO test precision often has been used to suggest precision of the BOD result.

DO precision is a relatively minor and controllable factor contributing to BOD results. Other factors such as organism suitability, members, adaptation and conditional variables are much more difficult to control or to evaluate.

- B The Analytical Reference Service report on Water Oxygen Demand, July, 1960 (Sample type VII) included the results of seeded samples of glucose-glutamic acid BOD results from 34 agencies on 2, 3, 5 and 7 day incubations.

The relative geometric standard deviation (average) was 19% on 2% sample and 24% on 1% sample concentrations. Rate coefficients ranged from 0.10 to 0.27 with a median of 0.16 from 21 different laboratories that participated in rate studies.

VI ALTERNATE BOD TECHNIQUES

Reaeration methods are becoming increasingly popular in order to approach more nearly the actual waste concentration in the receiving water. It is common to obtain "sliding" BOD results related to the concentration of waste in a series of dilutions of the same sample. This may result from greater possibilities for toxic effects at higher concentrations, or to a different selection of organisms and change in oxidation characteristics at low concentrations of sample. The most reliable estimate of stream behavior is likely to be from that dilution closest to the wastewater dilution in the receiving water.

- A Reaeration can be accomplished by the usual series techniques by dumping all of the remaining sealed bottles into a common

container when the residual DO reaches about 1.0 mg/l. After reoxygenation, the remaining bottles are refilled and a new initial DO determined. Subsequent dissolved oxygen depletions are added incrementally as a summation of the total oxygen depletion from the start of the test. If necessary, the reaeration technique may be performed several times but at a sacrifice of double DO determinations for each day on which reaeration occurs.

- B Special methods of reaeration have evolved to minimize the extra manipulation for reaeration of individual sample dilutions.

1 Elmore Method

A relatively large volume of the sample is stored in an unsealed bottle. Small bottles are withdrawn in sets of 5 or more, sealed, incubated, and the DO determined at appropriate intervals. When the DO concentration in the smaller bottles reaches 1.0 mg/l, a new set is withdrawn from the large unsealed bottle, after reoxygenation if necessary.

2 Orford Method

The deoxygenation is carried out in a large sealed jug from which samples for DO are withdrawn at appropriate intervals. To maintain the waste level and a sufficient DO in the jug, additional waste is added from a second open container. See diagram.

- C Excess oxygen may be provided by oxygenation with commercial oxygen instead of with air to increase the initial oxygen content for incubation while limiting the number of dilutions or reaeration steps. When oxygen is used in place of air the oxygen saturation in water at 20°C is about 40 mg/l instead of 9 mg/l. Limited results are available hence the analyst must verify his technique. The DO tends to decrease as soon as the bottle is opened hence, about 35 mg/l of oxygen content is the top of the practical working concentration. There has been no evidence that the biota is inhibited by the higher oxygen content with respect to BOD progression.

- D Reaeration or Oxygenation Advantages and Limitations.

- 1 Reaeration expands the range of BOD results obtainable directly at field concentrations, but is not advisable for applications when the sample BOD exceeds 50 mg/l.

- 2 Dilution water problems are eliminated, to the extent that the stream sample may be tested without dilution.
- 3 Incubator storage space becomes a real problem for multiple sample routine.

VII Dissolved oxygen electrodes, polarographic and others, are feasible for use in BOD determinations, often making it possible to make an estimate of DO or BOD when sample interference prevents a valid Winkler DO determination.

Electronic probe DO makes it possible to determine many successive DO's at different time intervals on the same bottle with negligible sample loss. Reaeration or extended time series, therefore, are more feasible.

Another outline in this series describes response of reaerated BOD₅ with electronic DO probes.

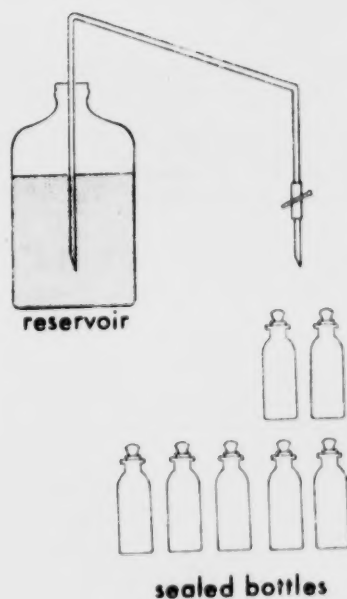
A It is the responsibility of the analyst to evaluate:

- 1 Applicability of the specified technique and sample.
- 2 To determine requirements for mixing and possible thermal effects while mixing in terms of instrument response and biochemical reaction.
- 3 To evaluate long-term calibration or standardization and their effects upon precision and accuracy of the BOD result.

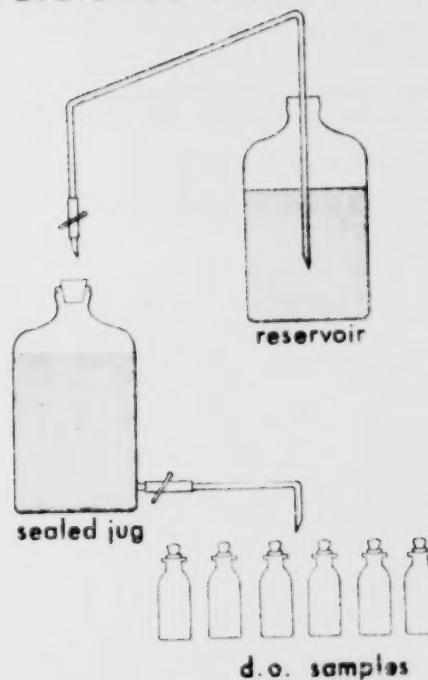
VIII IMMEDIATE DISSOLVED OXYGEN DEMAND (IDOD)

Immediate dissolved oxygen demand includes dissolved oxygen utilization requirements of substances such as those in I-A-3. An arbitrary period of 15 min. has been selected to distinguish IDOD from BOD.

REAERATION METHODS FOR B.O.D. DETERMINATION



ELMORE METHOD



ORFORD METHOD

A The IDOD is an apparent response as indicated by a specified technique. Since DO titration is based upon iodine titration, any factor that causes I_2 response different from that produced by the reaction of KI and molecular oxygen confuses the IDOD determination.

B IDOD Determination

- 1 The IDOD determination includes the determination of DO on a sample and dilution water separately. A waste likely to have a significant IDOD is unlikely to show a DO.
- 2 According to mixing theory, it should be possible to calculate the DO of any definite mixture of the sample and dilution water from the DO of component parts and their proportion. (See IV. E. 1).
- 3 The same relative proportions of sample and dilution water should be mixed without air entrainment and the DO determined after 15 minutes.
- 4 Any difference between the calculated initial DO as obtained in VIII-B-2, and the determined DO in VIII-B-3 may be designated as IDOD.
- 5 Sample aeration, DO interference, and other factors confuse results for IDOD.

C Sample Calculation of IDOD

- 1 Sample DO checked and shown to be 0.0 mg/l

Dilution water DO found to be 8.2 mg/l

Assume a mixture of 9 parts of dilution water and 1 part (V/V) of sample.

Calculated DO =

$$1 \times 0 = 0$$

$$9 \times 8.2 = 73.8$$

10 parts of the mixture contain 73.8/10 or 7.4 mg DO/l. Note that mixing has reduced the DO concentration because the original amount is present in a larger package.

- 2 The mixture described in C-3 was held for 15 minutes and the DO determined was 4.3 mg/l.

$$IDOD = DO_{calc} - DO_{detm} \times \frac{100}{\% \text{ sample used}}$$

$$= 7.4 - 4.3 \times 10$$

$$= 31 \text{ mg IDOD/l}$$

Bibliography

- 1 Standard Methods, APHA-AWWA-WPCF 13th Ed. 1971.
- 2 Methods for Chemical Analysis of Water and Wastes, EPA-AQCL. 1971.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, DTTB, MDS, WPO, EPA, Cincinnati, OH 45268.

BOD DETERMINATION - REAERATED BOTTLE PROBE TECHNIQUE

I INTRODUCTION

- A Customary dilution technique has certain limitations with respect to the BOD determination such as a prior commitment on sample dilutions to be used, number of bottles to be included to permit Winkler DO determinations to be made throughout a predetermined test interval, (with sample destruction for each DO test), possibility for anomalous effects due to dilution water, dilution, or inconsistent response between test bottles.

- B DO probe technique offers the advantages of nondestructive DO testing, possibilities of adjusting routine according to sample behavior during test, and retaining the sample with minor losses for long term observation (if desired) and for supplementary tests at the end of the BOD test interval.

Note: Use of the probe results in a loss of about 1/2 ml of each 300 ml sample. Use dilution water with a glass rod or bead to bring the sample volume back to 300 ml for further testing.

- C This test was performed in triplicate on the same sample at 100% concentration with reaeration as needed to illustrate results obtainable.

II PROCEDURE

A Sample

Final effluent (catch sample at 1100 hours) of the Advanced Waste Treatment Research Activated Sludge (Unit B) Sanitary Engineering Center Experimental Wing (September 3, 1970). One hundred percent concentration.

B DO Probe Calibration

The probe was calibrated daily in air saturated tap water versus the azide

modification of the iodometric titration. A replicate test bottle of the titrated sample was retained for initial probe adjustment, if necessary, and to recheck sensor response at the end of the use period. Zero sensor response in cobalt treated sulfite solution was checked at three day intervals. Electronic response and battery condition were checked daily.

C Reaeration

- 1 When the DO test indicated 1.0 mg DO/liter or appeared likely to reach that point prior to the next test reading the test bottles were reaerated and a new initial DO was obtained.
- 2 An adapter was inserted into the neck of the sample bottle indicating low DO and an empty bottle inserted on the opposite end. The combination was shaken vigorously, while in an inverted position to allow the transfer of sample to the empty bottle; immediately after transfer the combination was reinverted, shaken as before and sample returned to the original bottle.
- 3 The sample was allowed to stand for at least five minutes to allow entrained air to rise (generally formed a froth ring). The froth ring was raised into the neck of the bottle by displacement with about 1/2 ml of dilution water. It usually was necessary to tilt the bottle slightly and roll it to sweep fine air bubbles off the shoulder of the bottle and into the neck. After careful reinsertion of the DO sensor these bubbles were displaced by tipping the assembly and discarding contents of the BOD bottle lip containing the frothy residue.
- 4 It is recognized that surface active materials and possibly other components would concentrate in the discarded froth. It would be possible to limit this effect

by allowing a longer time for the froth to break--say 15 to 30 minutes. This was not attempted for this demonstration.

- D No attempt was made to determine DO at any regular time interval. Observation time is recorded for each test on a 24-hour clock basis with zero time at midnight.
- E Results are recorded in tabular form including the date (Column 1), time in hours (2), sample temperature (3), hours of incubation (4), and the triplicate sample data. For each of the samples the observed DO, the change since the last observation (ΔDO) and the summation of all the DO depletions observed during the incubated interval are recorded as $\Sigma \Delta DO$. A bold face line in the tabulated data in the DO column indicates DO before reaeration; the number below the line indicates DO after reaeration to be used as a new initial for the next observation.
- F Near the end of the fifth day of incubation (116 hours) one of the triplicates (no. 3) was not reaerated to check the effect of complete depletion of oxygen. It was reaerated on the following day and thereafter.
- G The results plotted in graphic form are presented following the BOD tabular data.
- H The results of a similar respiration test on mixed liquor from the same activated sludge units are tabulated to show the effects of different types of feed on DO depletion (respiratory activity). In this case, time is in minutes, DO is in mg/l and ΔDO /minute also is in mg/l. The first test in Table 2 represented 100 ml of return sludge plus plant effluent to fill the bottle. Approximately one percent nutrient agar solution was added by syringe (1.0 ml) to produce the response noted in the second series; one ml of five percent mercuric chloride produced the effects in series 3. A new replicate sample (100 ml sludge) and plant influent to fill the bottle produced the results in series 4.

III SUMMARY

- A Table 1 shows that it is possible to obtain consistent results using a DO probe plus reaeration for BOD technique. The major requirement is to carefully calibrate the DO instrument and sensor on a daily basis. It is not recommended to reaerate as many times as found necessary here. It could have been diluted at any time since the sample was available. Had the BOD response at five days been substantially complete only two reaerations would have been necessary. However, since the sample was working into second stage BOD it proceeded to react accordingly. The oxygen demand of 15 mg/liter at five days increased to 30 mg/liter in seven days and reached 45 mg/liter in ten days. The sample that was allowed to deplete on the fifth day slowly recovered but by the twelfth day or thereafter equaled or exceeded the BOD of the samples with residual DO at all times. This technique requires much less incubator space, time and manipulation with an added advantage of retaining the sample for adjustment or extended observation. The results do not depend upon a preconceived guesstimate which may or may not fit the situation.
- B Table 2 indicates the possibility for estimating feed acceptability in an activated sludge treatment plant. On many occasions an inquiry from an industrial plant, a different type of inflow or perhaps a new plant requires a decision regarding effects at the treatment plant. A respiration test by probe technique offers an opportunity to rate the situation within ten minutes after probe calibration. A test of sludge and effluent oxygen demand gives an estimate sludge respiratory activity now. When the same sludge is subjected to a new feed of an acceptable nature respiratory activity rises as in series 2. If the feed happened to be highly toxic as in series 3 (mercuric chloride) respiratory activity stops--

obviously a dead or severely shocked condition. A replicate sludge (Series 4) with the regular plant influent shows a prompt increase in activity. Oxygen requirements, permissible load ratio, acceptable feed to sludge ratios (return sludge adjustment) all may be estimated on the basis of DO tests related to overall plant performance.

- C The graphic of BOD versus time includes the COD--an estimate of first stage oxygen demand and the TKN for the composite effluent sample. If the total Kjeldahl nitrogen (TKN) is multiplied by its oxygen equivalent (4.57) then the COD plus TKN times 4.57 gives an estimate of ultimate oxygen demand (138 mg/l).

The twenty day BOD was about $55 \times 100/138$ or forty percent of this value. The effluent sample obtained at 1100 hours is unlikely to contain a significant portion of the high load period considering the primary, aerator and secondary clarifier detention. Also the COD and TKN may have included materials that were chemically oxidized but gave a poor response to biological oxidation. The BOD gives the rapid respiratory oxygen demand. COD plus the oxygen equivalent of N (on the same sample) gives a quick estimate of potential ultimate demand.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, DTTB, MDS, WPO, EPA, Cincinnati, OH 45268.

BOD Determination - Reaerated Bottle Probe Technique

TABLE 1
BOD Tabulated Results - Reaerated Sample; Probe DO
9/3/70 100% Sample

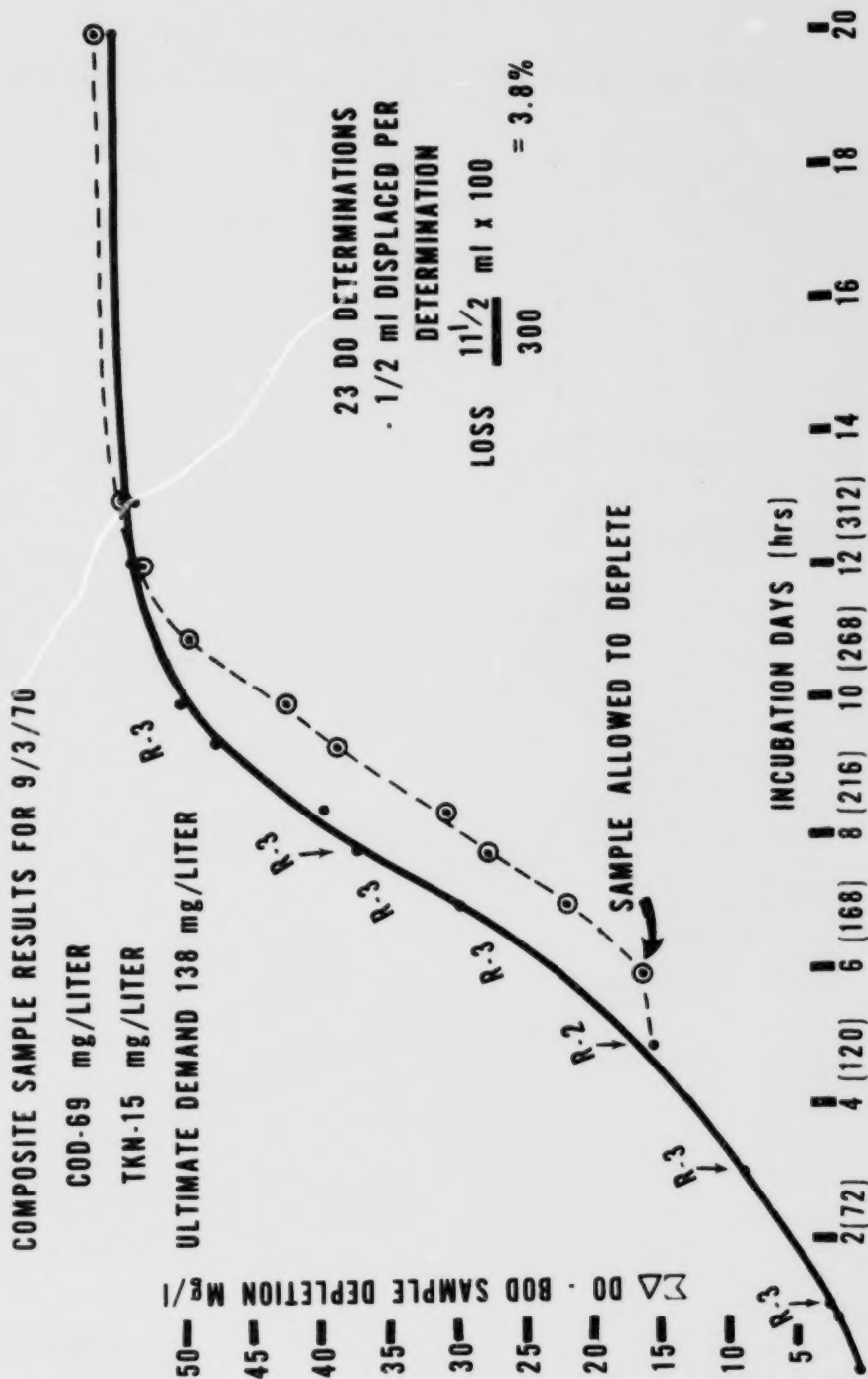
Date	Time	T°C	Hrs. Incub	Sample 1			Sample 2			Sample 3		
				DO	ΔDO	ΣΔDO	DO	ΔDO	ΣΔDO	DO	ΔDO	ΣΔDO
9/3/70	1340	24.0	0	6.7			6.7			6.8		
9/3/70	1355	24.0	0.25	6.7	-		6.7	-		6.8	-	
9/3/70	1620	22.0	2.6	6.5	0.2	0.2	6.5	0.2	0.2	6.6	0.2	0.2
9/4/70	1010	20.2	20.5	4.9	1.6	1.8	4.7	1.8	2.0	4.8	1.8	2.0
				8.2			8.3			7.9		
9/4/70	1600	20.6	26.3	7.4	0.8	2.6	7.5	0.8	2.8	6.9	1.0	3.0
9/6/70	1340	19.5	72	1.0	6.4	9.0	1.5	6.0	8.8	1.1	5.8	8.8
				8.1			8.1			7.4		
9/8/70	0940	19.8	116	0.6	7.5	16.5	0.5	7.6	16.4	0.4	7.0	15.8
				8.6			7.6					
9/9/70	1235	20.0	143	1.1	7.5	24.0	1.1	6.5	22.9	0.0	0.4	16.2
				8.2			8.2			7.6		
9/10/70	1200	20.0	166	1.0	7.2	31.2	1.2	7.0	29.9	2.0	5.6	21.8
				8.2			8.6			8.0		
9/11/70	0800	20.0	186	1.2	7.0	38.2	1.1	7.5	37.4	2.0	6.0	27.8
				8.4			8.3			8.5		
9/11/70	1400	20.0	192	5.0	3.4	41.6	5.3	3.0	40.4	5.7	2.8	30.6
				8.1			8.4			8.6		
9/12/70	2035	20.0	222	0.5	7.6	49.2	0.6	7.8	48.2	0.4	8.2	38.8
				8.2			8.2			8.2		
9/13/70	1130	20.0	238	6.3	1.9	51.1	3.8	4.4	52.6	4.6	3.6	42.4
				8.3			8.4			8.3		
9/14/70	1200	20.0	262	7.0	1.3	52.4	6.9	1.5	53.1	0.9	7.4	49.8
										8.3		
9/15/70	1340	19.6	288	5.7	1.3	53.7	6.2	0.7	53.8	4.5	3.8	53.6
										8.2		
9/16/70	1200	19.3	310	5.1	0.6	54.3	5.8	0.4	54.2	7.2	1.0	54.6
*9/20/70	1240	21.6	480	3.8	1.3	55.6	4.6	1.2	55.4	4.9	2.3	56.9

* Incubator power off

TABLE 2
ACTIVATED SLUDGE RESPIRATION DATA DO PROBE TECHNIQUE

Time Min	Temp	DO	Δ DO	Sample
0	24.0	7.2	Series 1	100 ml Return Sludge & Effluent
1		6.6	0.6	
2		6.2	0.4	
3		5.6	0.6	
4	24.5	5.1	0.5	
0		4.7	Series 2	+ 1 ml Nutrient agar
1		3.8	0.9	
2		2.7	1.1	
3		1.7	1.0	
0	24.8	4.7	Series 3	+ 1 ml HgCl ₂ Soln
1		4.8		
2		4.7	Nil	
3		4.7		
0	24.2	5.9	Series 4	100 ml Sludge + Influent
1		4.6	1.3	
2		3.3	1.3	
3		2.0	1.3	

GRAPHIC OF REAERATED PROBE BOD RESULTS
STARTING 9/3/70 SAMPLED 1100 HOURS



EFFECT OF SOME VARIABLES ON THE BOD TEST

I TIME

A The common equation $y_t = L(1 - 10^{-k_1 t})$ for BOD relationship indicates time as a variable. The rate coefficient (k_1) indicates that a specific percentage of material initially present (oxygen) will be used during a given time unit. Each successive unit of time has less reactant present initially than the preceding interval, hence a definite percentage decrease results in successively smaller amounts of reactant use per unit of lapsed time. Increasing k_1 results in a larger percentage oxygen use per unit of time and also increases the change in reactant mass among successive time intervals.

B Adney's work for the British Royal Commission cited 5 days passage time from source to the ocean as maximum for English streams. The 8th Report (1909) largely established BOD philosophy including the 5-day interval. At 5 days, initial lags generally have terminated and a substantial fraction of the long-term oxygen demand has been exerted. If only one time interval can be used, 7 days permits better scheduling. Any one time interval is "a" fraction of the total oxygen requirement; this is a poor reference point if we do not know how it arrived. For example, the percentage of oxidizable material stabilized in terms of oxygen use at various rate factors are:

k_1	% oxidized	K_1
$(\log_{10})$ in 5 days		$(\log_e)$
0.05	42%	0.11
0.10	67%	0.23
0.15	84%	0.34
0.25	94%	0.57
0.50	99+%	1.15

This range ($K_1 = 2.3 k_1$) is commonly encountered in wastewater stabilization with the higher rates characteristic of fresh oxidizable material that is readily converted.

The lower coefficients are characteristic of cell mass at later stages of oxidation and of low-rate reactants in general.

C The oxygen utilization at specified intervals of time are required to estimate k_1 , and L , the estimate of oxygen use at infinite time. It is common to observe results at equal intervals of time but this is not essential as long as the time intervals are accurately known. The initial time periods are critical as an error of a few hours in time represents a relatively large change in reactant mass in a system at maximum instability. Unequal time periods can be plotted to define the curve from which any given intervals can be selected as desired.

D Increasing impoundment of surface water provides more time for stabilization of relatively inert soluble or suspended pollutants and for organism adaptation to the situation or pollutants. Long term BOD's are essential to indicate changes in the pattern of oxygen demand vs. time. It may be expected that one or more plateaus will be evident in the BOD curve followed by a temporary rise in rate during second stage oxidation or thereafter. Anaerobiosis may cause a rise in rate coefficient after aerobic conditions are re-established. Eventually k_1 stabilizes at very low values.

1 Rate coefficients tend to be difficult to interpret during long term BOD's because of progressive changes and other factors.

a The relative error of the DO test may be a large fraction of the incremental DO change during low rate periods.

b Cell mass may agglomerate under quiescent test conditions and decrease nutrient availability.

c It is not likely that recycled nutrients under aerobic test conditions will have as much effect as recycle from anaerobic benthic deposits in a stream.

- 2 The BOD result tends to underestimate deoxygenation relative to surface water behavior because of interchanges, turbulence, biota, and boundary effects. Reseeding does not occur in a sealed bottle but reseedling is inevitable in a stream or treatment unit.

II TEMPERATURE

A Effect on Oxidation Rate

Temperature is one of the important controlling factors in any biological system. In the BOD reaction, changes in temperature produce acceleration or depression of the rate of oxidation. Figure 1 shows the changes in the value of k at temperatures from 0 - 25°C on a common wastewater.

B Test Temperature

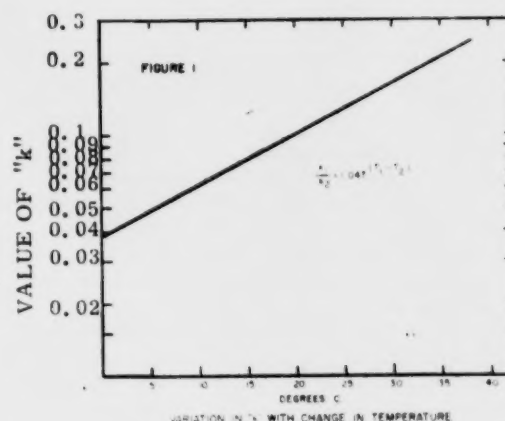
In the BOD test procedure an arbitrary temperature is usually selected for convenience even though a wide temperature range exists under natural conditions.

Incubation of the test containers at 20°C for the whole period is now accepted practice in the U.S.; 18.5°C is preferred in England. Camp (ASCE, SA5 91:1, Oct. 65) recommended light and dark bottle immersion in the stream.

C Temperature Correction

When it is necessary to calculate the rate of oxidation at a temperature other than 20°, the following relationship may be used:

$$\frac{k_1}{k_2} = \theta^{(T_1 - T_2)}$$



where:

k_1 = rate coefficient at temperature T_1

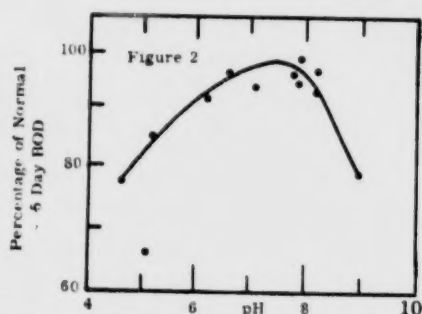
k_2 = rate at coefficient at temperature T_2

θ = temperature coefficient, for which Streeter and Phelps obtained the value 1.047. θ changes with temperature; it appears to be higher in the range of 5-15°C than in the range of 30 to 40°C. The value given refers to 15-30°.

The cited temperature coefficient appears reasonable for household wastes. It may not apply for other wastes where developing or seed organisms may not tolerate temperature changes as readily. A given temperature coefficient should be checked for applicability under specified conditions.

III pH

- A The organisms involved in biochemical conversions apparently have an optimum response near a pH of 7.0 providing other environmental factors are favorable; a pH range of about 6.5 to 8.3 apparently is acceptable (Figure 2). Reactivity is likely to be significantly lower on both sides of the acceptable pH range but microbial adaptation may extend the limits appreciably. For example, trickling filters have operated with better than 50% treatment efficiency at pH 3 and 10 after adaptation.



B Adjustment of Concentrated Samples

When wastes are more acid than pH 6.5 or more alkaline than pH 8.3, adjustment to pH 7.2 is advisable before reliable BOD values can be obtained.

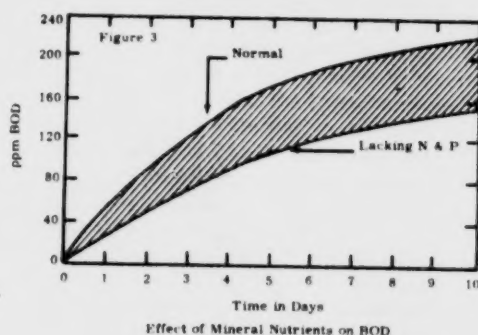
C Dilution Samples

Standard dilution water is buffered at pH 7.2. Sample-dilution water mixtures should be checked to make sure that the sample buffer capacity does not exceed the capacity of the dilution water for pH adjustment.

IV ESSENTIAL MINERAL NUTRIENTS

A Importance

In 1932 Butterfield reported on the role of certain minerals in the biochemical oxidation of sewage and concluded that deficient minerals often upset metabolic response. In addition, he found that inadequate nitrogen and/or phosphorus was a common cause of low BOD results in industrial wastewaters. (Figure 3)



B Standard Methods Dilution Water

The dilution water specified for the BOD test approximates USGS estimates for an average U.S. mineral content of surface water except for added phosphate buffer. It is assumed to provide essential mineral nutrients for most wastewaters but cannot be expected to meet requirements for grossly deficient wastewater nutrients both mineral and organic. Ruchhoff (S.W.J. 13:669, 1941) summarized committee action leading to the present dilution water.

C Other Dilution Considerations

There is a trend toward the use of receiving water, storage-stabilized if necessary, to evaluate waste behavior. It is advisable to minimize dilution and consider the nutrient level likely in the receiving water as most valid. Any change in the environment, such as dilution, upsets the microbial balance and requires adaptive changes.

V MICROBIOLOGICAL POPULATION

A Need for Complex Flora and Fauna

Butterfield, Purdy, and Theriault (Pub. Health Rep. 393, 1931) demonstrated that an isolated species of organisms was not as effective in biological stabilization as a variety of species. Figure 4 summarizes some of their data. Bhatta and Gaudy (ASCE, SA3, 91:63, June 1965) reinvestigated this factor. Many studies have emphasized the need for a mixed biota in the BOD test. It appears that bacteria are capable of varied activities, but all species are not capable of synthesizing all required nutrients. Certain bacterial species may be capable of producing enzymes, amino acids, or growth factors needed for their use and by other species for optimum performance. It has been shown that oxygen demand becomes minimal when some limit of bacterial population has been reached. Predation prevents such an approach to maximum numbers and maintains a continuing bacterial growth and recycle of nutrients among a mixed population. The net effect is a symbiotic relation among mixed organisms tending to enhance the rate of stabilization or utilization of oxygen as in the BOD test.

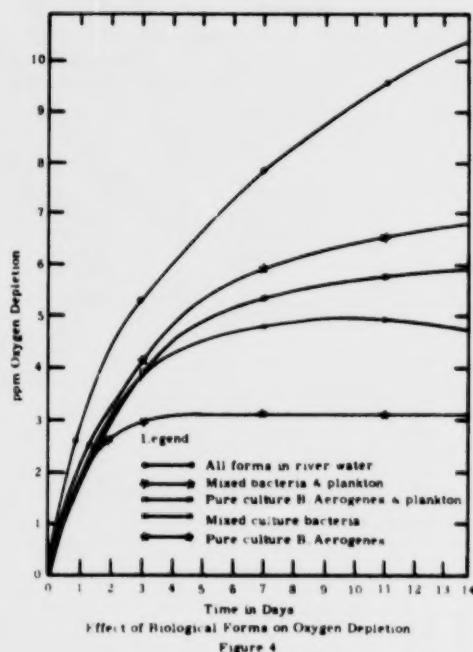
B Organism Adaptation

- 1 Early investigations in relation to the BOD test considered domestic wastewaters primarily. The saprophytic organisms involved in stabilization either were present in adequate numbers or quickly multiplied to attain effective populations.
- 2 The period of adjustment required to shift enzyme production needed to utilize an energy source different from that previously utilized or to shift population variety from that favored by one food to that favored by another food is considered an adaptation period. Dilution, temperature, oxygen tension, pH, nutrient type, inhibitory substances, light and other changes all are common inducements for microbial adaptation. Mutation of organisms may be encountered during adaptation but usually is not a factor.
- 3 The developments in industry and technology have resulted in discharge of new and more varied wastewater constituents. Microorganisms may adapt themselves to the use of a new substance as an energy source providing the energy and environment are favorable. The receiving stream usually shows development of an adapted microbiota for a new or different discharge constituent within hours, days or weeks after fairly regular discharge. The time for adaptation depends on the nature of the constituent, available energy, tolerance of the organisms, and environmental conditions.

C Seeding

The amount of seed and its selection must be determined experimentally. The most effective inoculant would be that which would produce the maximum BOD response with minimum lag period and negligible seed demand. This would mean some maximum population adapted to feed and conditions at a minimum equilibrium energy nutrient supply.

- 1 Figure 5 indicates corrected BOD progression on a synthetic feed with river water and stale sewage inoculants at several concentrations. The river water resulted in higher BOD with negligible lag and seed correction. The seed correction at 20% concentration of inoculant was less than 0.3 mg. DO/l at 5 days. It would be possible to use this river water as a diluent without excessive oxygen loss to produce more valid BOD progression for that receiving water. The lower wastewater inoculant concentration resulted in a definite BOD lag. Higher wastewater concentrations produced comparable BOD progression earlier but resulted in high seed corrections and lowered availability of dissolved oxygen for the sample.
- 2 A good secondary treated effluent produced results similar to river water inoculation with higher seed corrections per increment of applied inoculant. Soil suspensions also are very effective sources of seed organisms with minor seed corrections if they are reasonably stabilized surface soils.
- 3 It appears that the BOD progression most nearly indicating receiving water oxidation would be one based upon receiving water dilution or inoculated with organisms from it.
- 4 A new or unusual wastewater may require adapted organisms not present in sufficient numbers in the receiving water. Development of an adapted seed from soil suspensions, plant effluents or receiving water may be necessary to evaluate oxidation potential in a plant or receiving water at some future time. Enrichment culture technique is beneficial where small concentrations of the test wastewater are applied regularly with increases in wastewater concentrations as BOD or respiration activity indicates increasing tolerance and oxidation of the test waste. Both time and concentration limits are useful to characterize the wastewater and its acceptability for biological stabilization.



Effect of Biological Forms on Oxygen Depletion
Figure 4

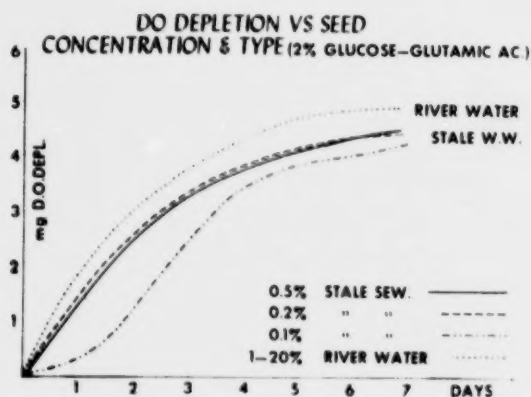


Figure 5

- 5 It must be recognized that BOD progressions are most likely to err on the low side. A meaningful BOD test should seek the highest consistent oxygen demand feasible for sample and conditions.

D Algae

When large numbers of algae are present in surface waters, they produce significant changes in the oxygen content. Under the influence of sunlight excess oxygen is produced while a net deficit occurs in the dark. The result is a wide variation in surface water DO depending on sample time.

When stream samples containing algae are incubated in the laboratory the algae survive for a time, then die because of the lack of light. Short-term BOD determinations may show the influence of oxygen production by the algae. When the algae die, they release the stored organic load for recycle and increase the BOD. Therefore, samples incubated in the dark may not be representative of the deoxygenation process in the stream, since the benefits of photosynthesis are lacking. Conversely, samples incubated in the light, under conditions of continual photosynthesis, will yield low BOD values.

The influence of algae on BOD is one of the most difficult variables to evaluate. More research is needed to develop satisfactory methods for the accurate determination of BOD in the presence of large numbers of algae. Light and dark bottle incubations suggest the magnitude of effects.

IV TOXICITY

A Effect

Since satisfaction of the BOD is accomplished through the action of microorganisms, the presence of toxic substances will result in depression of the oxidation rate. In many cases, toxicity will produce a lag period, until tolerant organism activity becomes significant. Figure 6 shows the effect of cyanide on the BOD curve. A prominent lag period is exhibited in the 2 ppm curve, while at 10 ppm the lag extends beyond the fifth day. An activated sludge may be adapted to work effectively in degradation of 60 mg CN/l.

Effect of Some Variables on the BOD Test

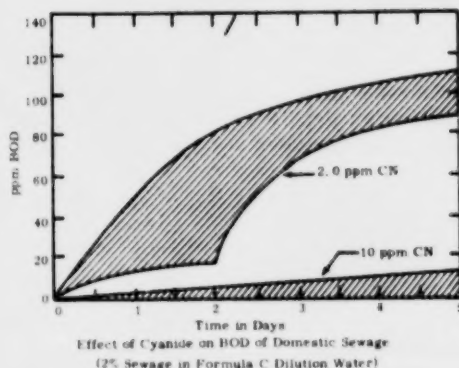


Figure 6

Heavy metals have similar effects depending on history and environment. The effects of copper and chromium are illustrated in Figure 7.

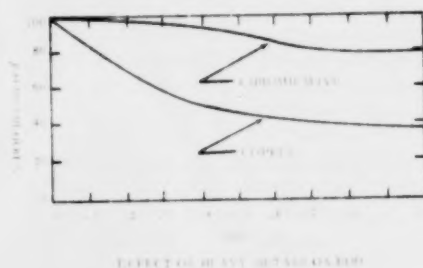


Figure 7

B Detection

In laboratory determinations of BOD the absence of toxic substances including chlorine must be established before the results can be accepted as valid. Comparison of BOD values for several dilutions of the waste will indicate the presence or absence of toxicity. In Table I the calculated BOD for the dilutions show higher values in the more dilute concentrations. It is apparent that toxicity was present and that the toxic effect was diluted out at a waste concentration of 2% or less.

Table I

Waste conc.	Depletion	5 day BOD
10%	3.51	35
5%	4.53	91
2%	2.80	140
1%	1.52	152
0.5%	0.74	148

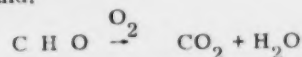
VII NITRIFICATION

A Mechanism

The oxidation process, as exemplified by the equation:

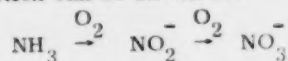
$$y = L(1 - 10^{-kt})$$

presumably involves the oxidation of carbonaceous matter or 1st stage oxygen demand.



The rate coefficient is normally high, giving nearly complete oxidation in a few days.

When nitrogenous material is present its oxidation can be shown as:



Nitrogen oxidation may be delayed for several days during BOD tests unless suitable micro-biota are initially available. Under some circumstances these two oxidations can proceed simultaneously and the resultant BOD curve will be a composite of the two reactions.

$$y_t = \left[L_c (1 - 10^{-k_c t}) + L_n (1 - 10^{-k_n t}) \right]$$

where y_t = the simultaneous BOD of the carbonaceous and nitrogenous phases or 1st and 2nd stage demands.

k_c and k_n = the rate coefficients applicable to the carbonaceous and nitrogenous materials respectively.

L_c and L_n = the ultimate oxygen demands characteristic of the two phases respectively. This is the general formula for any system characterized by two simultaneous reactions.

Principal conditions governing simultaneous carbon and nitrogen oxidation:

- 1 Presence of an effective nitrifying culture at the beginning of the test interval (nitrifiers grow relatively slowly).
 - 2 Maintenance of adequate DO, believed to be a minimum of 0.5 to 1.0 mg/l, for nitrifier activity.
 - 3 Available nitrogen - in excess of that required for synthesis. This is believed to require a minimum of about 7 mg/l to support active nitrification on a continuous basis.
 - 4 Nitrifiers appear to be more sensitive to toxicity than most saprophytic organisms, hence are likely to be inhibited more readily. This is particularly evident during nitrite to nitrate conversion.
- B It may require 5 to 10 days to establish nitrification if the population was not nitrifying initially. This is the basis for the sequential carbonaceous and nitrogenous oxidation of sewage oxidation.
- 1 Effects on the BOD curve indicate a typical pattern such as in Figure 8. The influence of nitrification in the production of a secondary rise in the BOD curve is so well known that any secondary rise may be erroneously attributed to nitrification whether or not nitrification was involved. Actually, a secondary rise in the curve may be due to any oxidation system assuming dominance after the initial oxidation system has been completed.
 - 2 The nitrification phenomena occurs simultaneously in many streams, treated effluents or partially stabilized samples. The designation of a secondary

BOD rise to nitrification should be based on analysis, not curve shape.

- C The extent of nitrification is conclusively shown only by periodic analysis of ammonia, organic, nitrite and nitrate nitrogen. The conversion of ammonia and organic nitrogen to oxidized nitrogen is a definite indication of nitrification.

D Nitrification Inhibition

Plant efficiencies from a BOD standpoint can be erroneous because nitrification generally is not established during the usual incubation of influent samples but may be a major factor in effluent incubations. It requires about 2 times the oxygen to convert $\text{NH}_3\text{-N}$ to $\text{NO}_3\text{-N}$ as to convert C to CO_2 hence this is a major fraction of stream oxygen use. Most secondary treated effluents are characterized by a larger fraction of carbon than nitrogen removal which accentuates the problem.

Pasteurization of samples, methylene blue, chromium, and acid treatment followed by neutralization have been used to inhibit nitrification for estimation of carbonaceous BOD only. Any inhibition of nitrification also produces a change in the sample or its behavior and may partially inhibit carbonaceous oxidation. Nitrification is a factor in stream self-purification and treatment. It does not appear realistic to alter it for convenience. The most realistic approach to carbonaceous oxidation is the measurement of CO_2 or COD.

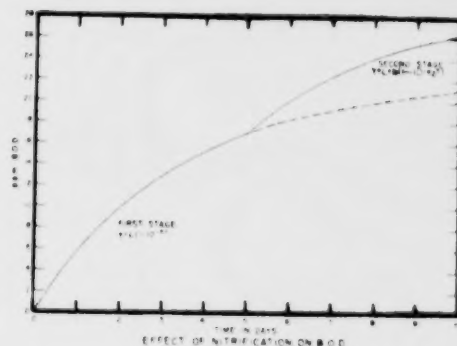


Figure 8

VIII EFFECT OF DILUTION

When a series of dilutions are made on a BOD sample usually the results vary to the extent that only an approximate BOD value is obtained.

Table 2
INTERPRETATION OF BOD DATA

Sample conc.	DO	Depletion	BOD
Initial	8.2	-	-
Final:			
1%	5.5	2.7	270
2%	3.3	4.9	245
4%	0.0	Complete	-

A For example, in Table 2, 1%, 2% and 4% concentrations of sample were used. The 4% concentration became anaerobic before the end of 5 days. The 5-day BOD of the 1% concentration was 270 and that of the 2% concentration was 245.

B Statistically one value is more reliable than the other.

Dilution	DO depletion
1%	5.5 mg/l
2%	3.3 mg/l
Difference	2.2 mg/l

The difference in depletion between 1 and 2% dilutions is 2.2 mg/l. This difference may be attributed to an additional 1% of sample added to the original 1%. If the difference is multiplied by the dilution factor of 100 to obtain the BOD, the result is 220 mg/l.

1 We now have three estimates of the BOD on a one percent concentration basis from the two dilutions:

- a the actual 1% depletion gives 270
- b 2%/2 depletion gives 245
- c (2% - 1%) depletion gives 220

Statistically the probabilities of being nearer the actual value goes with the nearest two of three. The 4% value of 8.2 depletion/4 as a minimum possible BOD 1% concentration gives a BOD of at least 200.

2 There is the possibility that higher concentrations may reflect significant toxicity while lower concentrations tend to reflect a greater proportion of dilution water. The toxicity problem does not appear to be significant since the 4% sample concentration indicated a BOD of at least 200. The higher BOD at 1% sample concentration may be due to a contaminated dilution water or to the fact that a similar number of seed organisms had less food and utilized certain fractions that they had passed by when they had more choice with the 2% sample concentration. Data is insufficient to resolve this one.

3 Incubations having a depletion of at least 2 mg DO/liter and a residual of at least 1 mg DO/liter are indicated to be most valid⁽¹⁾. Both the 1 and 2% concentrations fit this requirement in Table 2. An average error of + or - 0.1 ml on the DO titration would have a smaller relative error upon the 2% depletion.

4 We have a reasonable presumption that the sample BOD of about 230 was a good estimate. We do not have an unequivocal basis for so stating. Possible variations in results with different dilutions of a given sample are subject to many uncertainties in the test routine.

If some cause is known - such as a titration error, the inclusion of extraneous substances producing high or low response, or a definite procedural error that rules out a valid estimate of the sample BOD- that result should be labeled as a lost cause or unreported. Otherwise, report what was obtained to the best of your ability with the provision of uncertainty for uncontrollables.

ACKNOWLEDGMENT:

Certain portions of this outline contain training material from prior outlines by D. G. Ballinger and J. W. Mandia.

REFERENCE:

Standard Methods, APHA-AWWA-WPCF,
13th edition, 1971.

This outline was prepared by F. J. Ludzack,
Chemist, National Training Center, DTTB,
MDS, WPO, EPA, Cincinnati, OH 45268.

MATHEMATICAL BASIS OF THE BIOCHEMICAL OXYGEN DEMAND (BOD) TEST

Part 1

I FUNDAMENTAL CONCEPTS

For a number of years, the oxidation of organic waste substances in surface waters has been under investigation. The first reported observation of oxygen depletion was on the Seine River below Paris (1877). Other studies followed in Germany, England and the U.S. Certain fundamentals are now universally accepted. These are:

- A Dissolved oxygen in the water is reduced during stabilization of the organic material.
- B As long as dissolved oxygen is present, the rate of oxidation is independent of the actual amount of oxygen available.
- C The type and numbers of biological forms present is an important factor.
- D Measurement of changes in oxygen content can be related to quantity and character of oxidizable organic matter.

II FIRST ORDER RATE CONCEPT

In order to illustrate the mathematical relationships in the BOD reaction, assume the following laboratory observations:

- A A set of replicate bottles is filled with river water and sealed so that outside air is excluded.
- B Each day, one of the bottles is analyzed for DO content, and the results tabulated. (See Table 1.)

It will be noted that on each successive day, the DO concentration is less than the day before. That is, oxygen is being consumed by biological or chemical action in the water. The oxygen residual when plotted against time forms the deoxygenation curve (Figure 1).

The oxygen demand or BOD curve is represented by the summation of observed oxygen depletions vs time (Figure 2). This

Table 1.

Days	DO	Oxygen depletion	
		Per day	Cumulative
0	9.2	0	-
1	7.4	1.8	1.8
2	5.8	1.6	3.4
3	4.6	1.2	4.6
4	3.7	0.9	5.5
5	2.9	0.8	6.3
6	2.3	0.6	6.9
7	1.8	0.5	7.4
8	1.5	0.3	7.7
9	1.2	0.3	8.0
10	0.9	0.3	8.3

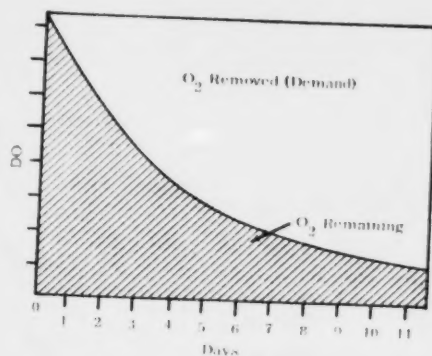


Figure 1

is an inverted representation of the de-oxygenation curve. Inclusion of the daily equivalents of oxygen demand in Figure 2 shows that increasingly smaller amounts of oxygen normally are used during each successive day.

If the reaction follows a 1st-order rate system, a constant percentage of the oxygen present at the beginning of each day will be used during that day. Therefore, the rate coefficient (K in nat. log. or k_1 in $\log_{10}$) is constant from day to day. $K_1 = 2.3 k_1$.

Similarly, the organic matter present in the sample is being oxidized, so there is progressively less oxidizable material present each successive day. The relationship between oxygen demand and the amount of oxidizable material present can be stated as follows: The oxygen demand per unit time is proportional to the amount of unoxidized material present. Streeter and Phelps^(1,2) stated the concept as - the rate of biochemical oxidation of organic matter is proportional to the concentration of unoxidized substance, measured in terms of oxidizability. Such a rate is termed a First Order Reaction Rate. The same authors also stated that there is no logical reason to expect one rate coefficient but it may appear so because of crude measurement and the effects of many individual oxidation systems.

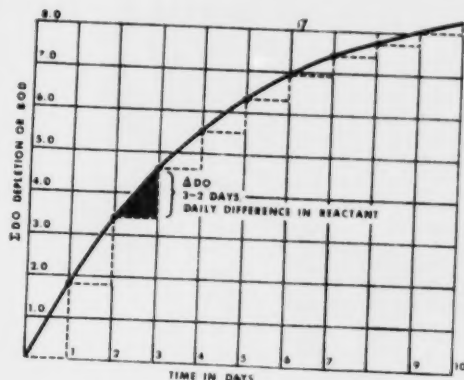


Figure 2. INTEGRAL BOD CURVE

The curve as shown in Figure 2 is a typical BOD curve. When interpreted in light of the fundamentals previously discussed, it is apparent that:

- 1 With organic materials which are easily oxidized, the reaction proceeds rapidly, nearing stabilization in a few days. The curve is therefore steep, rapidly approaching a maximum.
- 2 As oxidation nears completion the BOD curve will approach a limit or maximum value that can be represented by a line above the time axis and parallel to it. This line represents the ultimate oxygen demand (L) for the sample and conditions of the test. The curvature of the BOD line as it approaches the maximum is a function of the rate of oxidation.
- 3 The BOD test commonly is applied for samples containing mixed components each of which may differ in availability as a nutrient. The population of organisms generally contains many varieties. A progression of varieties occurs from those favored by an excess of initial nutrients to those favored by cell mass and eventually to those tolerating a low available energy level.

4 This type of system may have apparent characteristics of 1st order reaction mechanisms for limited periods of time. It is likely that two or more 1st order mechanisms may be apparent if observation time, nutrients and conditions are appropriate. The data also may be interpreted as a zero order, 2nd order or multiple order reaction mechanism. It is not always possible to prove reaction order nor is it essential to do so. The main objectives are to be able to estimate the ultimate BOD, the amount of BOD remaining after a given time interval and to obtain reasonable agreement among observed and calculated data. The first order reaction concept provides "a" means to meet the need without implying that the reactions are necessarily 1st order or that it is the only means of approach.

III BOD EQUATION

Using the typical BOD reaction curve, it is possible to develop equations expressing the various relationships. Labeling the coordinates as in Figure 3.

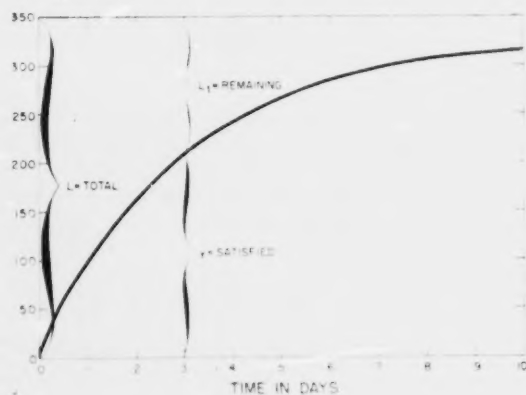


Figure 3

t = time in days
 L = ultimate demand
 L_t = demand remaining at time t
 y = demand satisfied at time t

then:

$$\frac{L_t}{L} = \text{fraction of } L \text{ remaining at time } t$$

$$\frac{y}{L} = \text{fraction of } L \text{ oxidized at time } t$$

The relationships may be expressed as a differential equation with respect to "t"

$$\frac{dL}{dt} = -KL \quad (1)$$

where K is a rate coefficient for deoxygenation as a $\log_e$.

$$\ln \frac{(L-y)}{L} = -Kt \quad (2)$$

changing to $\log_{10}$

$$\log_{10} \frac{(L-y)}{L} = -kt \quad (3)$$

and:

$$\frac{(L-y)}{L} = 10^{-kt}$$

Table 2
Equivalents in the BOD Equation

BOD remaining at time t	$L-y$	L_t	
Fraction of BOD remaining	$1 - y/L$	L_t/L	10^{-kt}
Conversion of $\log_e$ to $\log_{10}$	K	$2.3k$	
Fraction of BOD satisfied	$1 - \frac{L-y}{L}$	$1 - \frac{L_t}{L}$	$1 - 10^{-kt}$

from Table 2

$$1 - \frac{L-y}{L} = 1 - 10^{-kt} \quad (4)$$

multiplying both sides of equation (4) by L:

$$L \left(1 - \frac{L-y}{L} \right) = L(1 - 10^{-kt})$$

rearranging and cancelling gives:

$$y = L(1 - 10^{-kt}) \quad (5)$$

This is the usual form of the BOD equation. To avoid confusion with other "k" values, the deoxygenation rate is usually written as k_1 and the equation becomes.

$$y = L(1 - 10^{-k_1 t}) \quad (6)$$

The components in Figure 5 are as in III where L is the ultimate oxygen demand at infinite time and y refers to the demand satisfied at time t. The term k_1 refers to a "velocity constant" in physical chemistry terminology. Section II B3 indicates factors that result in system changes during progression of the BOD test that may affect the value of k_1 . For this reason the term "rate coefficient" is used for k_1 to recognize that k_1 may not be a "constant" except for limited time periods. As given, k_1 is an expression of the slope dL/dt as a log and is related to the rate of oxidation.

The L value is a laboratory number regarded as a theoretical rather than an actual limit. The limit is useful for an estimation of the effects of a waste upon the receiving water where the total demand is more valid than a part of it as for example a 5 day BOD.

IV SIGNIFICANCE OF BOD CONSTANTS

A The effect of k_1 upon y is shown in Figure 4.

The upper curve represents the BOD of a domestic sewage. The k_1 is 0.15.

At this rate, 50% of the demand will be exerted in the first two days, indicating that the organic matter is readily available as food for the organisms. In five days, 83% of the demand has been satisfied and in 15 days, the oxidation is essentially complete.

The lower curve represents the BOD of a relatively stable sample. The k_1 in this case is 0.05. In five days only 44% of the demand is satisfied and at 15 days, there is still 17% of the material unoxidized. Note that at the lower rate, it takes 15 days to accomplish 83% oxidation, while at the higher rate, the same percentage can be accomplished in 5 days.

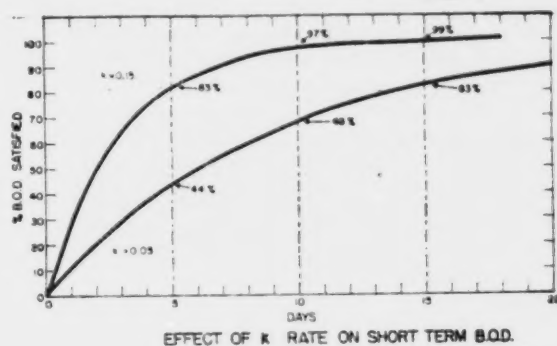


Figure 4

Laboratory tests on the BOD of waste materials are generally 5-day tests. In the case of these two samples, the standard 5-day interval would represent vastly different degrees of oxidation.

V LOG OF % BOD REMAINING

Using the mathematical relationships established above, a second BOD equation can be developed.

From Table 2:

$$\frac{L - y}{L} = 10^{-k_1 t} = \text{fraction remaining}$$

$$10^{-k_1 t} \times 100 = \% \text{ remaining}$$

$$\log_{10} (10^{-k_1 t} \times 100) = \log_{10} \% \text{ remaining}$$

further:

$$\log_{10} (10^{-k_1 t} \times 100) = 2 - k_1 t$$

then:

$$\log \text{ of } \% \text{ BOD remaining} = 2 - k_1 t \quad (7)$$

VI SUMMARY

The relationships presented herein form the basis for development of engineering estimates from laboratory data. Validity of the estimate depends upon sampling, prompt and correct BOD technique, judicious data analysis, and realization that the laboratory BOD test does not include many factors controlling stream behavior.

Two examples of the estimation of k and L values are presented in Part 2, which begins on the next page.

ACKNOWLEDGEMENT:

Certain portions of this outline contain materials made available by D. G. Ballinger, F. P. Nixon and D. E. Baumgartner.

REFERENCES

- 1 Streeter, H. W., and Phelps, E. B. Public Health Bulletin 46, USPHS. 1925.
- 2 Phelps, E. B. Stream Sanitation. John Wiley & Sons, New York. 1944.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, DTTB, MDS, WPO, EPA, Cincinnati, OH 45268.

MATHEMATICAL BASIS OF THE BIOCHEMICAL OXYGEN DEMAND (BOD) TEST
ESTIMATION OF k_1 and L

Part 2

I Several methods are available that may give a fair estimate of k_1 and L providing that the observed data plots in a form that suggests a first order reaction rate fit. The fact that stream samples frequently consist of partially stabilized river water mixed with varying amounts of more recent wastewater additions leads to numerous situations where observed data calculated as "a" first order reaction does not give a good fit of observed and calculated data. The low rate oxidation of the aged material and high rate oxidation of the new material simply cannot be explained in terms of one k_1 and L .

The BOD test is not precise enough to distinguish the infinite number of individual rate systems included in deoxygenation of a mixed wastewater by the mixed organisms involved. The persistence of the one first order reaction rate concept (incorrectly called a monomolecular rate) partially is due to the practical limitations of the test. High assimilative oxidation rates generally have been partially completed in the sewer, during compositing or sample storage before BOD analysis, or in the receiving water. Remaining deoxygenation may take the form characteristic of cell mass and storage products, (endogenous oxidation) which for a few days shows a k_1 of about 0.1. If the observation period was extended beyond the usual 7 to 10 days for rate estimation a progressive change of k_1 would become apparent as the more oxidizable components disappear and relatively inert biological or other residues became a larger and larger fraction of the total oxidizable mass. The increased impoundment of surface water makes long-term deoxygenation more and more significant.

The computed k_1 and L are a result of the selected measurement routine, past history of the sample, and the manner of processing the result. The validity of the estimate depends upon engineering judgment and a realization of the variables and effects involved. No two individuals will derive

precisely the same values from given data but common sense approaches using mathematics as a tool rather than as a deity, will produce useful estimates for resolution of the problem.

II Useful methods of deriving k_1 include the least squares method of Reed and Theriault (Public Health Bulletin 127, 1927); the Thomas Slope Method (Sew. Works J. 9, No. 3, 425, 1937), the Moments Method of Moore, Thomas, and Snow (Sew. and Ind. Wastes 22, No. 10, 1343, 1950) and the Rapid Ratio Method of Sheehy (JWPCF 32, No. 6, 646, 1960). The first three of these presume a single rate coefficient and do not give a good fit of data when deoxygenation fails to follow the pattern. **Certain other factors enter the picture even when the curve apparently follows first order characteristics** (Ludzack et al, Sew. and Ind. Wastes 25, No. 8, 875, 1953). Sheehy's procedure elucidates the disappearance of high rate components during early stages and can be very useful for rapid calculations.

The daily difference method outlined by Tsivoglou (Oxygen Relations in Streams, SEC Tech. Report W-58-2, p. 151, 1958) is an adaptation of Fair and of Velz methods and gives a graphic picture of observed data and predominant rate changes with time. The method is rapid, versatile, and gives a great deal of information in a simple package that is readily assembled. This technique was used to illustrate its applicability as "one" means of estimation.

A Using the data given in Table 1, Part 1.

- 1 Plot y vs. t on cartesian coordinate paper. Draw a curve of best fit through the observed points, including lags, plateaus, if any, to get a picture of the deoxygenation curve. If a majority of observed values describe a smooth

progression, those that fail to fit may be considered erratic and curve values may be used for subsequent operations. Lags should be eliminated by curve fitting and taking the observed points after the lag termination. (Figure 2)

- 2 Plot the daily differences, corrected if necessary, on semilog paper with time on the linear scale and the daily difference on the log scale. The differences are conventionally plotted as 1/2, 1-1/2, 2-1/2 days, etc. to illustrate intervals rather than points. (Figure 5)
- 3 Examination of Figure 2 shows identical daily differences for the last 3 days or $k_1 = 0$ for that interval in Figure 5. That is unlikely considering the short period of observation and the results of the first 6 days.
- 4 It may be assumed that deoxygenation follows a smooth curve after considering possible lag periods or multicomponent rates operating in combination or sequentially. Daily differences, therefore, should be calculated from a line of best fit such as Figure 2 rather than from the individual points included in Table 1 which may be affected by experimental error.
- 5 Figure 5 is a plot of the daily differences from Table 1 on the $\log_{10}$ scale vs time on the arithmetic scale. The corrected curve of best fit was not used here to show the effects of the last 3 points on the logarithmic pattern. The slope of the line was well defined on the basis of the first 7 points. Note that the daily differences are plotted at the middle of the time interval, i.e., the deoxygenation occurring from 0 to 1 day appears at the 1/2 day position and that occurring during the second day appears at the 1-1/2 day position.

- 6 It is advisable to check arithmetic on the work sheet by comparing the sum of the daily differences in Table 1 with that resulting from the line of best fit in Figure 5. In Table 1: $1.8 + 1.6 + 1.2 + \dots + 0.3 = 8.3$; in Figure 5; $1.88 + 1.50 + 1.20 + \dots + 0.26 = 8.4$. Since the difference in sums is 1 part in 84 it is apparent that serious errors did not occur in arithmetic or plotting.

- 7 From Figure 5, the number at the zero intercept of the daily difference slope is a function of L. Other intercepts are a function of L_t using equivalents in Table 2. The number representing L at 0 time = 2.1; the number representing L_t when $t = 10$ is 0.23. The percent BOD remaining when $t = 10$ is $L_t/L \times 100$ or $\frac{0.23 \times 100}{2.1} = 10.9$ or 11%.

- 8 From equation 7:

$$\log_{10} \text{ of the \% BOD remaining} = 2 - k_1 t$$

$$\text{The Log}_{10} \text{ of } 11\% = 1.04 \text{ and } t = 10 \\ \text{then } 1.04 = 2 - k_1 \times 10$$

$$\text{or } k_1 = 2 - 1.04/10 = \frac{0.96}{10} = 0.096 \text{ or } 0.10$$

- 9 From equation 6:

$$y_t = L (1 - 10^{-k_1 t}) = L (1 - \frac{L_t}{L})$$

$$\text{From Table 1, } y_{10} = 8.3, \text{ and}$$

$$(1 - \frac{L_t}{L}) = 0.89 \text{ (Step 7)}$$

The estimate of L becomes

$$y_{10} = L (1 - \frac{L_t}{L}) \text{ or } 8.3 = L (0.89)$$

$$L = \frac{8.3}{0.89} = 9.34 \text{ or } 9.3 \text{ mg BOD/l.}$$

- 10 The laboratory test gives estimates of the rate coefficient or k_1 of 0.10 and of L or the Ultimate Oxygen Demand of 9.3 mg of BOD per liter of sample. The data approximates a system consisting primarily of the oxidation of partially stabilized cell mass and storage products and plots as a one rate system during the observation period.

Equations 6 and 7 provide the fundamental relationships and the equivalents in Table 2 may be read from Figure 5.

- B The data in Table 3 present a different situation involving more recent contamination.

Table 3

days	BOD	per day
1	2.24	2.24
2	3.18	0.94
3	3.81	0.63
4	4.22	0.41
5	4.56	0.34
6	4.88	0.32
7	5.12	0.24
8	5.32	0.20
9	5.49	0.17
10	5.61	0.12

- 1 The line of best fit of the observed BODs in Table 3 are shown as a smooth curve in Figure 6.
- 2 Figure 7 shows the daily differences on the semi log scale vs the time interval on the arithmetic scale. The line ab is a reasonable fit for the slope described by 5 of the 7 daily differences after the 3rd day. It is apparent that the curve in Figure 6 cannot be described as one first order reaction.

The differences among the first 3 observed daily differences and the line ab are 1.55, 0.36 and 0.14 from Figure 7. If the differences are plotted on Figure 7, (coded as x) a new rate cd can be estimated that gives a reasonable fit with 2 of the 3 intervals. The first daily difference is above the slope cd suggesting a 3rd rate group higher than ab or cd . Since this would be based upon a single point in 1 series, in which some discrepancy is apparent in Figure 7 with respect to line fitting, it is advisable to regard it as a possibility as shown by the graphic but withhold judgement on the 3rd rate group for further evidence. The curve in Figure 6 may be approximated by a two rate system operating simultaneously with one of the two nearly completed in 3 days.

- 3 Mathematically this situation may be expressed as:

$$Y_t = L_{cd}(1 - 10^{-k_{cd}t}) + L_{ab}(1 - 10^{-k_{ab}t}) \text{ or}$$

$$= L_{cd}\left(1 - \frac{L_t}{L_{cd}}\right) + L_{ab}\left(1 - \frac{L_t}{L_{ab}}\right)$$

where $\frac{L_t}{L}$ may be obtained from

Figure 7 at the designated time intervals for each slope.

- 4 From Figure 7 the zero and 10 day intercepts of line ab are 0.75 and 0.13.

$$\text{The } \frac{\%L_t}{L} = \frac{0.13 \times 100}{0.75} = 17.4\% \text{ BOD}$$

remaining when $t = 10$.

The $\log_{10}$ of 17.4 is 1.24.

If the $\log_{10}$ of the % BOD remaining = $2 - k_1t$

then, $1.24 = 2 - k_{ab} \times 10$ and $k_{ab} =$

$$\frac{2 - 1.24}{10} = \frac{0.76}{10} = 0.076$$

- 5 From Figure 7 the zero and 2 day intercepts of line cd are 1.40 and 0.22.

$$\% \frac{L_t}{L_{cd}} = \frac{0.22 \times 100}{1.40} = 15.7\% \text{ BOD}$$

remaining when $t = 2$. The $\log_{10}$ of 15.7 is 1.20 as in Step 3:

$$k_{cd} = \frac{2 - 1.20}{2} = \frac{0.80}{2} = 0.40$$

- 6 From the relation in B2, L_{cd} may be approximated by substituting the observed value for the 2 day BOD in Table 2 or 3.18. The fraction of $\frac{L_t}{L_{ab}}$

from zero to 2 days is $\frac{0.53}{0.75}$ or 0.71

and $(1 - \frac{L_t}{L_{ab}}) = 0.29$, therefore the

use of the 2 day observed value for line cd is incorrect because 0.29 L_{ab} is included in the observed value.

Since L_{ab} is unknown, the first estimate of L_{cd} will be incorrect by the value of 0.29 L_{ab} but may be approximated later.

If $Y_{2cd} = L_{cd}(1 - \frac{L_t}{L_{cd}})$ where $Y_2 = 3.18$

and $\frac{L_t}{L_{cd}} = \frac{0.22}{1.40}$ then a high approximation of L_{cd} becomes:

$$3.18 = L_{cd} \times 0.84$$

$$L_{cd} = \frac{3.18}{0.84} \text{ or } 3.8 \text{ mg BOD/l.}$$

- 7 A similar approximation of L_{ab} may be set up for $t = 10$ where the observed Y_{10} from Table 2 of 5.6 is corrected by subtracting L_{cd} which should be almost complete by 10 days. Y_{10ab} becomes $5.6 - 3.8 = 1.8$ and

$$\frac{L_t}{L} = \frac{0.13}{0.75} \text{ or } 0.174.$$

Substituting the appropriate numbers as in step 5:

$$L_{ab} = \frac{1.8}{0.826} = 2.18 \text{ or } 2.2 \text{ mg/l.}$$

- 8 A better approximation of L_{cd} is now possible assuming that the 2 day observed value included the following:

$$Y_2 = 3.18 - 0.29 \times 2.2 \text{ or } 3.18 - 0.64 = 2.54$$

and

$$L_{cd} = \frac{2.54}{0.84} = 3.0 \text{ mg BOD/l instead of } 3.8.$$

A second approximation of L_{ab} would raise the estimate of L_{ab} because

$$Y_{10ab} = 5.6 - 2.9 = 2.7 \text{ and}$$

$$L_{ab} = \frac{2.7}{0.826} = 3.28 \text{ or } 3.3 \text{ mg BOD/l}$$

and an estimate of the L values of the sample plotted in Figure 6 would be $2.9 + 3.3$ or 6.2 mg BOD/liter .

- C The substitution of the derived values for the appropriate and ultimate oxygen demand into the expression given in B2 should give values comparable to the observed BODs in Table 3 or Figure 6. Further, it should be possible to approximate curves for the individual rate systems to obtain daily vectors, the sum of which would compare with the observed points. For example:

$$Y_t = L_{cd}(1 - 10^{-k_{cd}t}) + L_{ab}(1 - 10^{-k_{ab}t})$$

Where $L_{cd} = 2.9$ and $k_{cd} = 0.40$

$L_{ab} = 3.3$ and $k_{ab} = 0.076$

and $10^{-k_{ab}t}$ may be approximated by $\frac{L_t}{L}$ from Figure 7. (See Table 2)

$$1 \quad Y_1 = 2.9(1 - \frac{0.56}{1.40}) + 3.3(1 - \frac{0.63}{0.75})$$

$$= 2.9 \times 0.60 + 3.3 \times 0.16$$

$$= 1.74 + 0.53 = 2.27. \text{ observed} = 2.24$$

$$2 \quad Y_2 = 2.9(1 - \frac{0.22}{1.40}) + 3.3(1 - \frac{0.53}{0.75})$$

$$= 2.4 + 0.9 = 3.3 \text{ observed} = 3.18$$

$$3 \quad Y_3 = 2.9(1 - \frac{0.01}{1.40}) + 3.3(1 - \frac{0.44}{0.75})$$

$$= 2.9 + 1.3 = 4.2 \text{ observed} = 3.81$$

$$4 \quad Y_5 = 2.9 + 3.3(1 - \frac{0.31}{0.75})$$

$$2.9 + 1.9 = 4.8 \text{ observed } 4.56$$

Estimation of k and L

$$\begin{aligned} 5 \quad Y_{10} &= 2.9 + 3.3 \left(1 - \frac{0.13}{0.75}\right) \\ &= 2.9 + 2.7 = 5.6 \quad \text{observed } 5.61 \end{aligned}$$

- 6 These data show a reasonable approximation of the observed BODs by a reverse calculation from the derived rate and ultimate BOD obtained from them. The agreement indicates that approximations made were reasonably correct and that the composite BOD curve in Figure 6 may be described as a 2 rate system as indicated by the

BODs in Figure 6. The largest error occurred in calculation of the components for Y_3 at the point in time where the higher rate system was being phased out. Curve fitting, approximations of correction factors for estimation of L , plotting, slide rule and other errors contribute to estimates of k_1 and L in addition to the errors related to the BOD test.

An approximation of k_1 is possible from 2 BOD values obtained at different intervals of time by using the data presented in the Theriault Tables or that given nomographically by the chart on the Rates of BOD Satisfaction.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, DTTB, MDS, , EPA, Cincinnati, OH 45268.

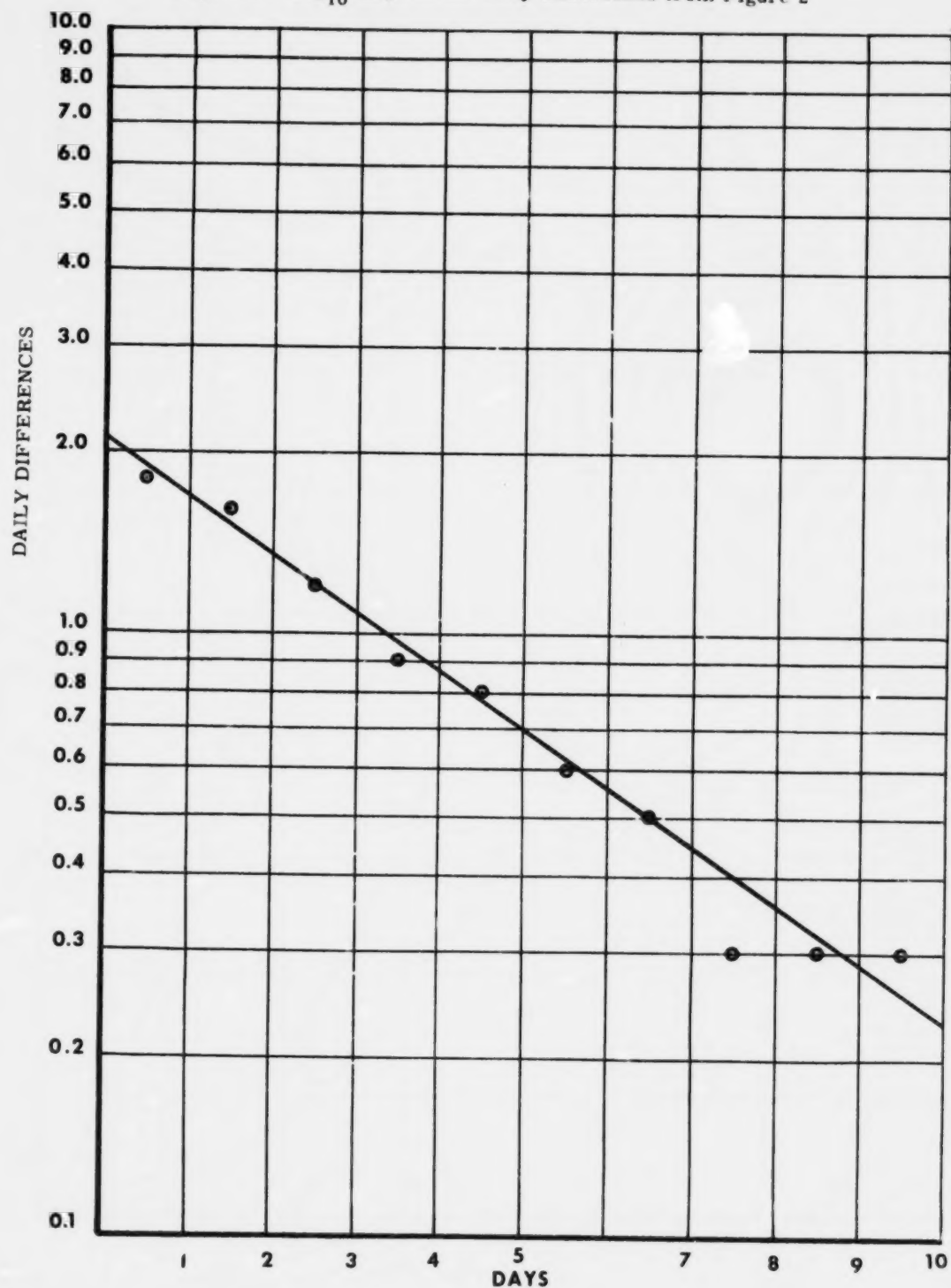
Figure 5. $\log_{10}$ Slope of the Daily Differences from Figure 2

Figure 6. Σ BOD of the Data from Table 2.

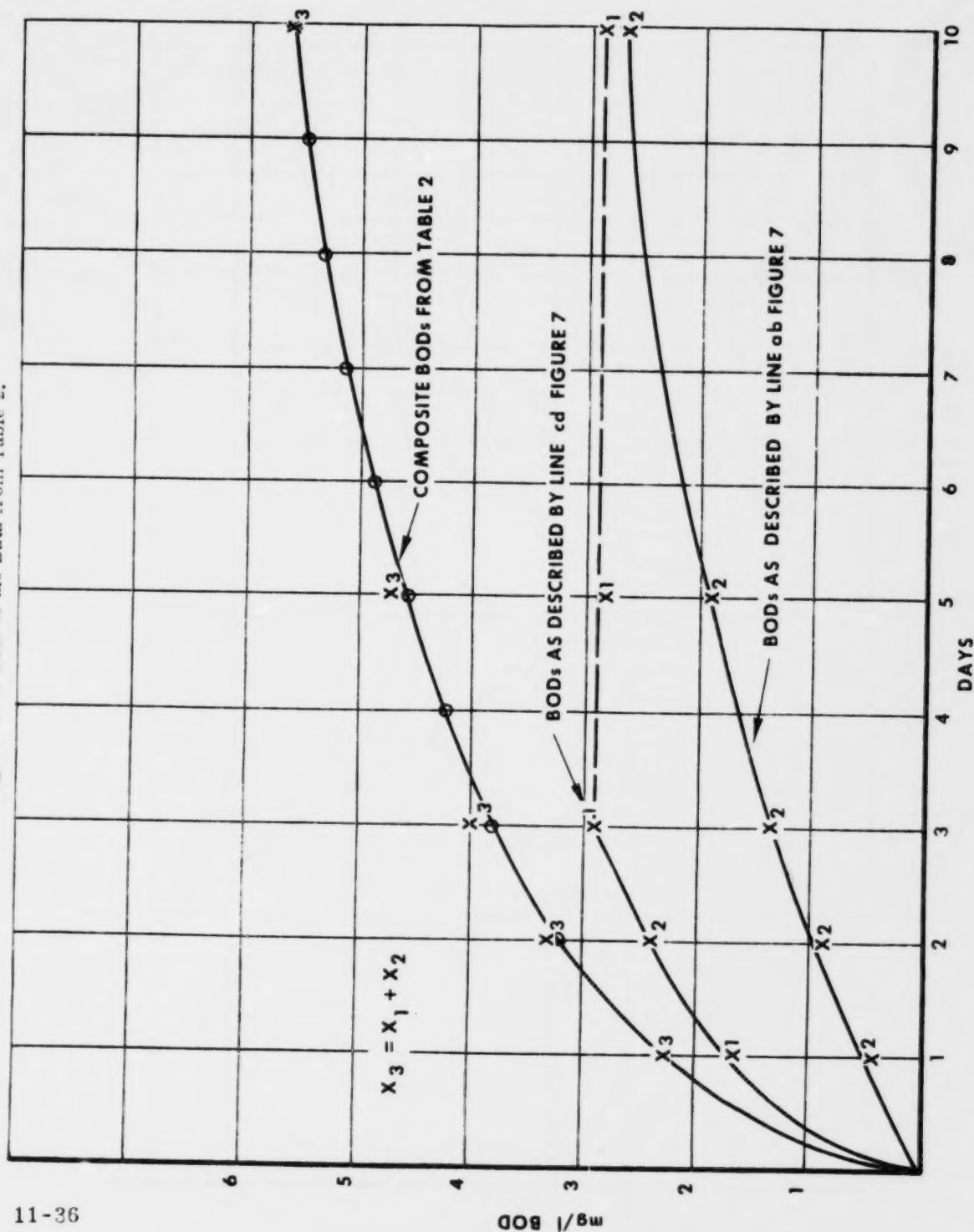
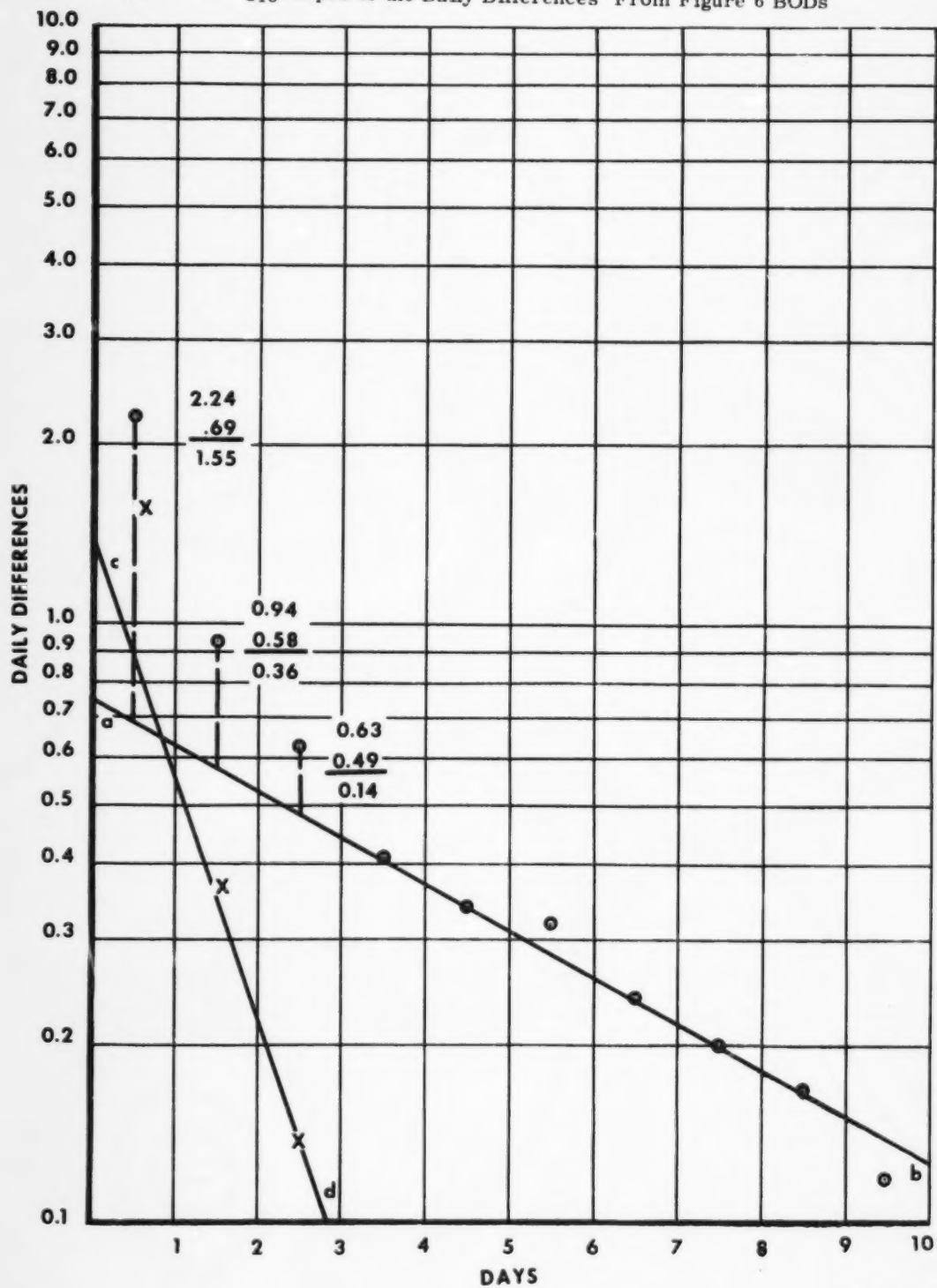


Figure 7. Log₁₀ Slopes of the Daily Differences From Figure 6 BODs



Theriault Table

NUMERICAL VALUES OF THE FUNCTION $(1 - 10^{-kt})$ FOR THE RANGE $k = 0.040$ to $k = 0.250$

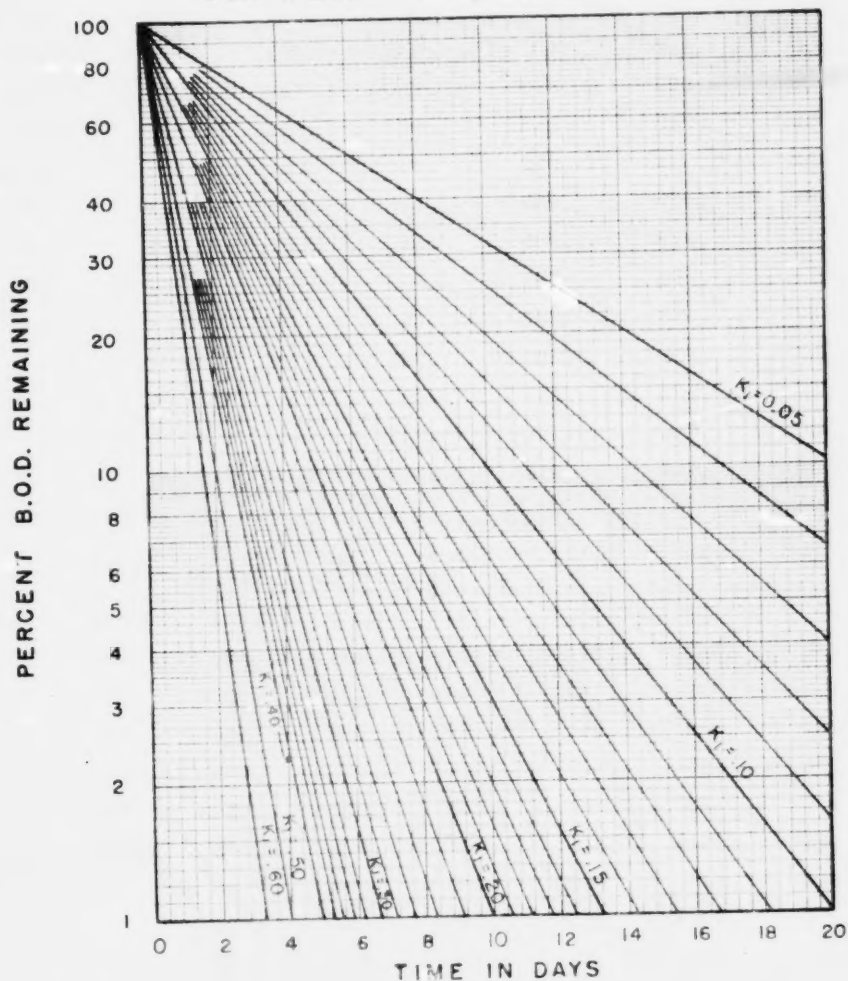
Period of incubation (days)	Value of k						
	0.04	0.05	0.06	0.07	0.08	0.09	0.10
1-----	0.088	0.109	0.129	0.149	0.168	0.187	0.206
2-----	.168	0.206	.241	.276	.308	.339	.369
3-----	.241	0.292	.339	.383	.425	.463	.499
4-----	.308	.369	.425	.475	.521	.563	.602
5-----	.369	.438	.499	.553	.602	.645	.684
6-----	.425	.499	.563	.620	.669	.712	.749
7-----	.475	.553	.620	.676	.725	.766	.800
8-----	.521	.602	.669	.725	.771	.809	.842
9-----	.563	.645	.712	.766	.809	.845	.874
10-----	.602	.684	.749	.800	.842	.874	.900
11-----	.637	.718	.781	.830	.868	.898	.921
12-----	.669	.749	.809	.855	.890	.917	.937
13-----	.698	.776	.834	.877	.909	.932	.950
14-----	.725	.800	.855	.895	.924	.945	.960
15-----	.749	.822	.874	.911	.937	.955	.968
16-----	.771	.842	.890	.924	.948	.964	.975
17-----	.791	.859	.905	.935	.956	.970	.980
18-----	.809	.874	.917	.945	.964	.976	.984
19-----	.826	.888	.928	.953	.970	.981	.987
20-----	.842	.900	.937	.960	.975	.984	.990
21-----	.855	.911	.945	.966	.979	.987	.992
22-----	.868	.921	.952	.971	.983	.990	.994
23-----	.880	.929	.958	.975	.986	.991	.995
24-----	.890	.937	.964	.979	.988	.993	.996
25-----	.900	.944	.968	.982	.990	.994	.997
Period of incubation (days)	Value of k						
	0.11	0.12	0.13	0.14	0.15	0.16	0.17
1-----	0.224	0.241	0.259	0.276	0.292	0.308	0.324
2-----	.397	.425	.450	.475	.499	.521	.543
3-----	.532	.563	.593	.620	.645	.669	.691
4-----	.637	.669	.699	.725	.749	.771	.791

Theriault Table

Period of incubation (days)	Value of k						
	0.11	0.12	0.13	0.14	0.15	0.16	0.17
5-----	.718	.749	.776	.800	.822	.842	.859
6-----	.781	.809	.834	.855	.874	.890	.905
7-----	.830	.855	.877	.895	.911	.924	.935
8-----	.868	.890	.909	.924	.937	.948	.956
9-----	.898	.917	.932	.945	.955	.964	.970
10-----	.921	.937	.950	.960	.968	.975	.980
11-----	.938	.952	.963	.971	.978	.983	.987
12-----	.952	.964	.972	.979	.984	.988	.991
13-----	.963	.972	.980	.985	.989	.992	.994
14-----	.971	.979	.985	.989	.992	.994	.996
15-----	.978	.984	.989	.992	.994	.996	.997
16-----	.983	.988	.992	.994	----	----	----
17-----	.987	.991	.994	.996	----	----	----
18-----	.990	.993	.995	.997	----	----	----
19-----	.992	.995	.997	.998	----	----	----
20-----	.994	.996	.997	.998	----	----	----

Period of incubation (days)	Value of k							
	0.18	0.19	0.20	0.21	0.22	0.23	0.24	0.25
1-----	0.339	0.354	0.369	0.383	0.397	0.411	0.425	0.438
2-----	.563	.583	.602	.620	.637	.653	.669	.684
3-----	.712	.731	.749	.766	.781	.796	.809	.822
4-----	.809	.826	.842	.855	.868	.880	.890	.900
5-----	.874	.888	.900	.911	.921	.929	.937	.944
6-----	.917	.928	.937	.945	.952	.958	.964	.968
7-----	.945	.953	.960	.966	.971	.975	.979	.982
8-----	.964	.970	.975	.979	.983	.986	.988	.990
9-----	.976	.981	.984	.987	.990	.991	.993	.994
10-----	.984	.987	.990	.992	.994	.995	.996	.997
11-----	.990	.992	.994	.995	----	----	----	----
12-----	.993	.995	.996	.997	----	----	----	----
13-----	.995	.997	.997	.998	----	----	----	----
14-----	.997	.998	.998	.999	----	----	----	----
15-----	.998	.999	.999	.999	----	----	----	----

RATES OF B.O.D. SATISFACTION
FOR VALUES OF K_1 FROM .05 TO .60



CHEMICAL OXYGEN DEMAND AND COD/BOD RELATIONSHIPS

I DEFINITION

- A The Chemical Oxygen Demand (COD) is an estimate of the proportion of the sample matter susceptible to oxidation by a strong chemical oxidant. The current edition of Standard Methods, ⁽²⁾ specifies organic material which is generally the situation but not necessarily applicable.
- B A variety of terms have been and are used for the test described here as COD:
- 1 Oxygen absorbed (OA) primarily in British practice.
 - 2 Oxygen consumed (OC) preferred by some, but unpopular.
 - 3 Chemical oxygen demand (COD) current preference.
 - 4 Complete oxygen demand (COD) misnomer.
 - 5 Dichromate oxygen demand (DOC) earlier distinction of the current preference for COD by dichromate or a specified analysis such as Standard Methods.
 - 6 Others have been and are being used. Since 1960, terms have been generally agreed upon within most professional groups as indicated in I-A and B-3 and the explanation in B-5.
- C The concept of the COD is almost as old as the BOD. Many oxidants and variations in procedure have been proposed, but none have been completely satisfactory.
- 1 Ceric sulfate has been investigated, but in general it is not a strong oxidant.
 - 2 Potassium permanganate was one of the earliest oxidants proposed and until recently appeared in Standard Methods (9th ed.) as a standard procedure. It is currently used in British practice as a 4-hr. test at room temperature.
 - a The results obtained with permanganate were dependent upon concentration of reagent, time of oxidation, temperature, etc., so that results were not reproducible.
 - 3 Potassium iodate or iodic acid is an excellent oxidant but methods employing this reaction are time-consuming and require a very close control.
 - 4 A number of investigators have used potassium dichromate under a variety of conditions. The method proposed by Moore at SEC is the basis of the standard procedure. ^(1,2) Statistical comparisons with other methods are described. ⁽³⁾
 - 5 Effective determination of elemental carbon in wastewater was sought by Buswell as a water quality criteria.
 - a Van Slyke ⁽⁴⁾ described a carbon determination based on anhydrous samples and mixed oxidizing agents including sulfuric, chromic, iodic and phosphoric acids to obtain a yield comparable to the theoretical on a wide spectrum of components.
 - b Van Hall, et al., ⁽⁵⁾ used a heated combustion tube with infrared detection to determine carbon quickly and effectively by wet sample injection.
 - 6 Current development shows a trend to instrumental methods automating

conventional procedures or to seek elemental or more specific group determination.

II RELATIONSHIP OF THE COD TEST WITH OTHER OXIDATION CRITERIA IS INDICATED IN TABLE 1.

A Table 1

Test	Test Temp. °C	Reaction time	Oxidation system	Variables
BOD	20	days	Biol. prod. Enz. Oxidn.	Compound, environment, biota, time, numbers. Metabolic acceptability, etc.
COD	145	2 hrs.	50% H ₂ SO ₄ K ₂ Cr ₂ O ₇ May be catalyzed	Susceptibility of the test sample to the specified oxidation
IDOD	20	15'	Diss. oxyg.	Includes materials rapidly oxidized by direct action, Fe ⁺² , SH.
Van Slyke Carbon detn.	400+	1 hr.	H ₃ PO ₄ HIO ₃ H ₂ SO ₄ K ₂ Cr ₂ O ₇ Anhydrous	Excellent approach to theoretical oxidation for most compounds (N-nil)
Carbon by combustion +IR	950	minutes	Oxygen atm. catalyzed	Comparable to theoretical for carbon only.
Chlorine Demand	20	20 min.	HOCl soln.	Good NH ₃ oxidn. Variable for other compounds.

B From Table 1 it is apparent that oxidation is the only common item of this series of separate tests.

- 1 Any relationships among COD & BOD or any other tests are fortuitous because the conditions of test tend to give results indicating the susceptibility of

a given sample to oxidation under specified conditions that are different for each test.

- 2 If the sample is primarily composed of compounds that are oxidized by both procedures (BOD and COD) a relationship may be established.

- a The COD procedure may be substituted (with proper qualifications) for BOD or the COD may be used as an indication of the dilution required for setting up BOD analysis.
- b If the sample is characterized by a predominance of material that can be chemically, but not biochemically oxidized, the COD will be greater than the BOD. Textile wastes, paper mill wastes, and other wastes containing high concentrations of cellulose have a high COD, low BOD.
- c If the situation in item b is reversed the BOD will be higher than the COD. Distillery wastes or refinery wastes may have a high BOD, low COD, unless catalyzed by silver sulfate.
- d Any relationship established as in 2a will change in response to sample history and environment. The BOD tends to decrease more rapidly than the COD. Biological cell mass or detritus produced by biological action has a low BOD but a relatively high COD. The COD/BOD ratio tends to increase with time, treatment, or conditions favoring stabilization.
- 4 COD results are available while the waste is in the plant, not several days later, hence, plant control is facilitated.
- 5 COD results are useful to indicate downstream damage potential in the form of sludge deposition.
- 6 The COD result plus the oxygen equivalent for ammonia and organic nitrogen is a good estimate of the ultimate BOD for many municipal wastewaters.

B Limitations

- 1 Results are not applicable for estimating BOD except as a result of experimental evidence by both methods on a given sample type.
- 2 Certain compounds are not susceptible to oxidation under COD conditions or are too volatile to remain in the oxidation flask long enough to be oxidized.

Ammonia, aromatic hydrocarbons, saturated hydrocarbons, pyridine, and toluene are examples of materials with a low analytical response in the COD test.

- 3 Dichromate in hot 50% sulfuric acid requires close control to maintain safety during manipulation.
- 4 Oxidation of chloride to chlorine is not closely related to BOD but may affect COD results.
- 5 It is not advisable to expect precise COD results on saline water.

III ADVANTAGES AND LIMITATIONS OF THE COD TEST⁽²⁾ AS RELATED TO BOD

A Advantages

- 1 Time, manipulation, and equipment costs are lower for the COD test.
- 2 COD oxidation conditions are effective for a wider spectrum of chemical compounds.
- 3 COD test conditions can be standardized more readily to give more precise results.

IV BACKGROUND OF THE STANDARD METHODS COD PROCEDURE

- A The COD procedure ⁽¹⁾ considered dichromate oxidation in 33 and 50 percent sulfuric acid. Results indicated preference of the 50 percent acid concentration for oxidation of sample components. This is the basis for the present standard procedure.

B Muers⁽⁶⁾ suggested addition of silver sulfate to catalyze oxidation of certain low molecular weight aliphatic acids and alcohols. The catalyst also improves oxidation of most other organic components to some extent but does not make the COD test universally applicable for all chemical pollutants.

C The unmodified COD test result (A) includes oxidation of chloride to chlorine. Each mg of chloride will have a COD equivalent of 0.23 mg. Chlorides must be determined in the sample and the COD result corrected accordingly.

1 For example, if a sample shows 300 mg of COD per liter and 200 mg Cl^- per liter the corrected COD result will be $300 - (200 \times 0.23)$ or $300 - 46 = 254$ mg COD/l on a chloride corrected basis.

2 Silver sulfate addition as a catalyst tends to cause partial precipitation of silver chloride even in the hot acid solution. Chloride corrections are questionable unless the chloride is oxidized before addition of silver sulfate, i.e., reflux for 15 minutes for chloride oxidation, add Ag_2SO_4 , and continue the reflux or use of HgSO_4 (D).

D Dobbs and Williams⁽⁷⁾ proposed prior complexation of chlorides with HgSO_4 to prevent chloride oxidation during the test. A ratio of about 10 of Hg^{++} to 1 of Cl^- (wt. basis) appears essential. The Cl^- must be complexed in acid solution before addition of dichromate and silver sulfate.

1 For unexplained reasons the HgSO_4 complexation does not completely prevent chloride oxidation in the presence of high chloride concentrations.

2 Factors have been developed to provide some estimate of error in the result due to incomplete control of chloride behavior. These tend to vary with the sample and technique employed.

E It is not likely that COD results will be precise for samples containing high chlorides. Sea water contains 18000 to 20000 mgCl^-/l normally. Equivalent chloride correction for COD exceeds

4000 mg/l. The error in chloride determination may give negative COD results upon application of the correction.

Incomplete control of chloride oxidation with HgSO_4 may give equally confusing results.

HgSO_4 appears to give precise results for COD when chlorides do not exceed about 2000 mg/l. Interference increases with increasing chlorides at higher levels.

F The 12th edition of Standard Methods reduced the amount of sample and reagents to 40% of amounts utilized in previous editions. There has been no change in the relative proportions in the test. This step was taken to reduce the cost of providing expensive mercury and silver sulfates required. Results are comparable as long as the proportions are identical. Smaller aliquots of sample and reagents require more care during manipulation to promote precision.

G The EPA Methods for COD

1 For routine level COD (samples having an organic carbon concentration greater than 15 mg/liter and a chloride concentration less than 2000 mg/liter), the EPA specifies the procedures found in Standard Methods⁽²⁾ and in ASTM⁽⁸⁾.

2 For low level COD (samples with less than 15 mg/liter organic carbon and chloride concentration less than 2000 mg/liter), EPA provides an analytical procedure⁽⁹⁾. The difference from the routine procedure primarily involves a greater sample volume and more dilute solutions of dichromate and ferrous ammonium sulfate.

3 For saline samples (chloride level exceeds 1000 mg/liter and COD is greater than 250 mg/liter), EPA provides an analytical procedure⁽⁹⁾ involving preparation of a standard curve of COD versus mg/liter chloride to correct the calculations. Volumes and concentrations for the sample and reagents are adjusted for this type of determination.

V The precision of the unmodified COD result shows a standard deviation of $\pm 4\%$ of the mean ⁽³⁾ on low chloride samples. Silver sulfate modified COD results are likely to show a standard deviation about twice that without catalysis, due to questionable chloride behavior. The determination of chloride frequently shows a coefficient of variation (s/x) of 10 to 15%, hence high chloride samples result in COD precision controlled more by chloride behavior than organic oxidation.

VI REMARKS PERTINENT TO EFFECTIVE COD DETERMINATIONS INCLUDE:

A Sample size and COD limits for 0.25 N reagents are approximately as given. For 0.025 N reagents multiply COD by 0.1. Use the weak reagents for COD's in the range of 5-50 mg/l, (low level).

Sample Size	mg COD/l
20 ml	2000
10 ml	4000
5 ml	8000

B Most organic materials oxidize relatively rapidly under COD test conditions. A significant fraction of oxidation occurs during the heating upon addition of acid. The color change of dichromate after acid addition indicates the approximate fraction of dichromate remaining. If the mixed sample color changes from yellow to green after acid addition the sample was too large. Discard without reflux and repeat with a smaller aliquot until the color after mixing does not go beyond a brownish hue. The dichromate color change is less rapid with sample components that are slowly oxidized under COD reaction conditions.

C Chloride concentrations should be known for all test samples and results interpreted accordingly.

D Special precautions advisable for the regular COD procedure and essential when using 0.025 N reagents include:

- 1 Keep the apparatus assembled when not in use.
- 2 Plug the condenser breather tube with glass wool to minimize dust entrance.

3 Wipe the upper part of the flask and lower part of the condenser with a wet towel before disassembly to minimize sample contamination.

4 Steam out the condenser after use for high concentration samples and periodically for regular samples. Use the regular blank reagent mix and heat, without use of condenser water, to clean the apparatus of residual oxidizable components.

5 Distilled water and sulfuric acid must be of very high quality to maintain low blanks on the refluxed samples for the 0.025 N oxidant.

ACKNOWLEDGEMENT:

Certain portions of this outline contain training material from prior outlines by R. C. Kroner, R. J. Lishka, and J. W. Mandia.

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See Next Page.

Chemical Oxygen Demand and COD/BOD Relationships

This outline was prepared by F. J. Ludzack,
Chemist, National Training Center, MDS,
WFO, EPA, Cincinnati, OH 45268.

V. NUTRIENTS, NITROGEN, CARBON,
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NUTRIENTS: THE BASIS OF PRODUCTIVITY

I INTRODUCTION

- A Nutrients of importance include macro-nutrients: those needed in large quantities, and micronutrients: those needed in small amounts.
- B These nutrients are important because they promote biological responses which may interfere with some desired use of the water by man.
- C Other factors (e.g. temperature, light) affect the use of these nutrients and should be considered in an evaluation of the effects of nutrients upon the ecosystem.

II Algae, bacteria, fungi and aquatic plants are the forms of life which nutrients affect most directly.

A Algae are of Several Types

- 1 Phytoplankton are small algae suspended in the water and form the basis of productivity in the aquatic environment.
- 2 Benthic algae are those forms anchored to substrates of rock and bottom materials.
- B Aquatic plants are of several types. In general they may be referred to as rooted or floating forms.
- C Heterotrophic bacteria are fungi which respond to organic nutrients introduced into water. Autotrophic bacteria may respond and grow due to inorganic nutrient sources.

III BIOLOGICAL LAWS

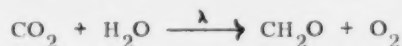
- A Liebig's "law" of the minimum: the essential material available in amounts most

closely approaching the critical minimum needed will tend to be the limiting factor.

- B Shelford's "law" of tolerance: survival of an organism can be controlled by the quantitative or qualitative deficiency or excess with respect to any one of several factors which may approach the limits of tolerance for that organism.
- C Q_{10} "law": with a temperature increase of 10 degrees centigrade metabolic processes (rates) are approximately doubled.

IV The process of photosynthesis is the fixation of the sun's energy with the production of organic matter by plants with chlorophyll.

- A The general reaction is given below:



- B Chlorophyll contains basically C, O, H, N and Mg, and in general makes up about 5% of the dry weight of algal cells.

V MEASUREMENT OF PHOTOSYNTHESIS

- A Oxygen production can be used as a measure of photosynthesis because for each mole of CO_2 reduced to organic carbon one mole of free oxygen is liberated.

- 1 The value of the molar O_2/CO_2 ratio has been found experimentally to vary within wide limits.

B CO_2 Assimilation

- 1 The CO_2 taken up by algae does not all originate from the dissolved gas. Some algae can use bicarbonate directly as a source of carbon.

- 2 Hence measurement of CO_2 uptake from water is a complicated problem which must consider pH, HCO_3^- , and CO_3^{2-} concentrations.

C Fixation of Carbon-14

- 1 The use of C^{14} as a tracer of C^{12} in plant metabolism and productivity estimation has been widely used since the early nineteen fifties.
- 2 In this method a known amount of C^{14} is added to the water and after a period of time the proportion of C^{14} in the plant cells to C^{14} added is found. The amount of carbon assimilated is then estimated from the following equation.

$$\frac{\text{activity of phytoplankton}}{\text{activity of } \text{C}^{14}\text{O}_3 \text{ added}} (K) = \frac{\text{total carbon assimilated}}{\text{total carbon available}}$$

- 3 Where K is a constant relating to the slower uptake of C^{14} .
- 4 The total carbon available is determined chemically.

D Uptake of Mineral Nutrients

- 1 The measurement of depletion of nutrients in solution has been tried but found unreliable.

E Chlorophyll

- 1 The quantity of chlorophyll present has been found to bear some relation to productivity but not a reliable one.

VI Nutrients of significance in the growth and production of algae and plants are discussed below.

A Carbon

- 1 Sources
 - a Gaseous CO_2
 - b HCO_3^-

c CO_3^{2-}

d Other carbon compounds

- 2 Effects of the removal of carbon upon the water
 - a Lowered pH
 - b Deposition of CaCO_3
- 3 The quantity of carbon available is great and it usually is not a limiting factor.

B Nitrogen

- 1 Nitrogen can be taken up by most algae as either ammonium salts or as nitrates. Nitrites can also be used but a high concentration is usually inhibitory. Some blue green algae can fix atmospheric nitrogen. Certain algae varieties require supplementary amines, growth factors, etc.
- 2 The quantity of nitrogen in waters has definitely been shown to limit algal populations.

C Phosphorus

- 1 Phosphate seems to be the only inorganic source of this nutrient.
- 2 Limiting concentrations of P have been found to range from .01 ppm at a minimum and an inhibitory affect if P concentrations exceed 20 ppm.
- 3 Optimum concentrations have been found to range from .018 ppm to 15 ppm.
- 4 Storage of inorganic phosphate by algae has been demonstrated. The extent of this storage may reach 80% of the total phosphorus in algal cells.

D Silicon

- 1 Nutrient ratios in the algal cells of some areas have been found to be Si 23: N 16: P 1. It can be seen from this ratio that silicon is an important element in algal growth.

- 2 Silicon is especially important in the population growth of diatoms and may be the limiting growth factor in these populations.

E Inorganic micronutrients - Many elements are needed in very small quantities by algal cells. Some of these have a known function in algal metabolism; others do not.

- 1 Mg is a cation of major importance in the chlorophyll molecule.
- 2 Co is known to be necessary for vitamin B₁₂.
- 3 Mn is necessary for several enzyme systems.
- 4 Mo, V, Zn, and Cu are necessary but these functions are not as well known.

F Organic Micronutrients

- 1 Of 179 algal strains investigated about 40% required vitamin supplementation for optimum growth. Principal growth factors that were not synthesized in sufficient quantity are given as follows along with the percentage of the vitamin deficient strains showing marked productivity gain after supplementation:
 - a B₁₂ addition increased growth on 80% of the strains.
 - b Thiamin addition increased growth on 53% of the strains.
 - c Biotin addition increased growth on 10% of the strains.
- 2 Algae can use and may require many organic compounds depending upon environmental conditions and the ability of the organism to synthesize required building blocks from mineral forms of C, N, & P. This is an area for continued investigation with many unappreciated or vaguely understood ecological factors.

VII PROBLEMS AND BENEFITS RESULTING FROM ALGAE PRODUCTION

A Problems to man may result when the total "primary production" by algae leads to an increase in the total organic content of the water that interferes with a desired use.

- 1 This may consist of a high algal population that produces a water with high turbidity, taste and odor, or other undesirable effect. High respiratory needs may lead to nocturnal oxygen deficit.

- 2 Certain algae may cause tastes and odors, clog filters; or otherwise interfere with potable water processing.

- 3 Death of large algal populations may lead to tastes and/or odors through bacterial decomposition. Oxygen deficits may result at any time of day in this process. Deposition of masses of organic sediment or sludge may be considerable.

- 4 Other problems might be cited.

B The primary production of algae can also serve as a supply of food to consumer organisms (animals), resulting in increased production at several (trophic) levels:

zoömicrobes, microinvertebrates, macroinvertebrates, fishes.

- 1 Earlier notation cited the release of oxygen during utilization of CO₂ during algal photosynthesis. This encourages fungal or bacterial breakdown of pollutants.
- 2 Photosynthesis occurs in the presence of adequate light and favorable conditions. In darkness, the cells continue to respire and may consume more oxygen than they produced because photosynthesis increases the organic load.

- 3 Photosynthesis tends to occur at the surface where light intensity is greatest. Poor vertical mixing would result in stratification of water supersaturated with oxygen over oxygen deficient water. Depending upon conditions, a significant fraction of the oxygen could be lost to the atmosphere.
- 4 Increased productivity may result in temporary reduction of the free dissolved nutrient level in the water but harvesting at some level is essential to prevent later recycle.

VIII CYCLE OF NUTRIENTS

- A Once nutrients enter a body of water they are cycled through a food chain.
- B Factors affecting this food chain (e.g. toxicity, removal) will affect the concentration and distribution of the nutrients.

ACKNOWLEDGEMENT:

This outline contains certain material submitted by F. J. Ludzack and H. W. Jackson.

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This outline was prepared by Michael E. Bender, Biologist, Formerly with Training Activities, Ohio Basin Region, SEC.

SOURCES AND ANALYSIS OF ORGANIC NITROGEN

I INTRODUCTION

- A Organic nitrogen refers to the nitrogen in combination with any organic radical. For sanitary engineering the main interest is the nitrogen contained in proteins, peptides, amines, amino acids, amides and related compounds of animal or vegetable origin.

II SOURCES OF ORGANIC NITROGEN

A Natural Origin

- 1 Dead animal and plant residues
- 2 Animal wastes - urea, feces
- 3 Autotrophic organisms - algae, s. bact.
- 4 Heterotrophic organisms

B Industrial Origin

- 1 Food processing wastewater - meat, milk, vegetables
- 2 Pharmaceutical wastes, antibiologics
- 3 Plastics - polyamides, nitriles
- 4 Chemical intermediates or products
- 5 Dye industry - azo, nitro

III TOTAL KJELDAHL NITROGEN PROCEDURE

- A Organic nitrogen is determined using the Total Kjeldahl Nitrogen (TKN) method. This determination includes both organic nitrogen and free ammonia. By distilling the free ammonia off the sample before the determination, organic nitrogen can be determined directly.

B Scope

The procedure converts nitrogen components of biological origin such as amino

acids, proteins and peptides to ammonia but it may not convert the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semi-carbazones and some refractory tertiary amines.

C Method Summary⁽¹⁾

The sample is heated in the presence of a concentrated sulfuric acid-potassium sulfate-mercuric sulfate mixture and evaporated until sulfur trioxide fumes are obtained and the solution becomes colorless or pale yellow. The residue is cooled and diluted, then treated and made alkaline with a hydroxide-thiosulfate solution. The ammonia is distilled off and then determined either by nesslerization or by titration.

- 1 Nesslerization (colorimetric) is the method used when the ammonia-nitrogen concentration is less than 1.0 mg N/liter.
- 2 For ammonia-nitrogen concentrations above 1.0 mg N/liter, the ammonia is determined by titration with 0.02 N H_2SO_4 in the presence of a mixed indicator.

For a detailed description of the procedure and reagent preparation, consult the EPA methods manual.

D Precision and Accuracy

- 1 Thirty-one analysts in 20 laboratories⁽¹⁾ used the Total Kjeldahl Nitrogen procedure to analyze natural water samples containing the following increments of organic nitrogen: 0.20, 0.31, 4.10 and 4.61 mg N/liter.
 - a Precision results for a standard deviation were 0.197, 0.247, 1.056 and 1.191 mg N/liter, respectively.
 - b Accuracy as bias was +0.03, +0.02, +0.04 and -0.08 mg N/liter, respectively.

- 2 The nature and composition of extraneous materials affect analytical recovery. High salt concentrations may raise digestion temperature. High concentrations of organic sample components may require excessive acid during digestion tending to low nitrogen yield.
 - 3 The digestion temperature is critical. 380 to 390°C usually gives high analytical recovery on the more refractory nitrogen compounds of natural origin. Nitrogen losses occur above 420°C.
- E Optimum temperature is associated with a digestion mix containing 1 gram of potassium sulfate for each ml of sulfuric acid. However, the digestion mixture usually contains a fraction of H_2SO_4 greater than this.

During heating, the water boils off first, followed by fuming as the H_2SO_4 becomes concentrated. Fuming and oxidation of the sample components lead to loss of H_2SO_4 and a progressive decrease in acid content during heating. As the acid decreases, the temperature of the heated mixture rises.

Valid nitrogen determinations require a slight excess of acid to retain NH_3 as NH_4HSO_4 rather than the more volatile $(NH_4)_2SO_4$. Concurrently, excess acid will tend toward incomplete oxidation of sample components as a result of lowering digestion temperature.

IV AUTOMATED PHENOLATE METHOD

The EPA manual⁽¹⁾ also presents an automated (phenolate) method for organic nitrogen. The sample is automatically digested with a sulfuric acid solution containing potassium sulfate and mercuric sulfate as a catalyst, then neutralized with sodium hydroxide solution and treated with alkaline phenol reagent and sodium hypochlorite reagent. This treatment forms a blue color designated as indophenol.

Sodium nitroprusside, which increases the intensity of the color, is added to obtain necessary sensitivity for measurement of low level nitrogen.

V PRESERVATION OF SAMPLES

- A Most nitrogen compounds are characterized by rapid conversion from one form to another by biological and chemical action. Hydrolysis, deamination, peptide formation, and other reactions may appreciably alter the original form of sample nitrogen within a short time. Organic nitrogen tends to hydrolyze to free NH_3 during storage. Therefore, valid results require prompt analysis.
- B Addition of 40 mg $HgCl_2$ /liter of sample and storage at 4°C are preservation measures for samples. Even when so preserved, samples are unstable and should be analyzed as soon as possible.

REFERENCE

- 1 Methods for Chemical Analysis of Water and Wastes, EPA-AQCL, Cincinnati, OH 45268 (1971).

This outline was prepared by Audrey E. Donahue, Chemist, National Training Center, DTTB, MDS, WPO, EPA, Cincinnati, OH 45268.

- 2 The digestate should be made alkaline with sodium hydroxide-thiosulfate solution without mixing. Connect the flask containing digestate and alkali to the distillation apparatus with the receiving tip well below the surface of the receiver containing 100 ml of 2% boric acid. Mix the alkalized digestion mix and distill until 200 ml of distillate have been collected.

V "FWQA Methods for Chemical Analysis of Water and Wastes, 1970" presents an automated phenolate method.

Organic nitrogen is determined by manually digesting the sample with potassium persulfate and sulfuric acid to convert the organic nitrogen, and any ammonia present, to ammonium sulfate. Subsequently, the automated phenol-hypochlorite procedure is used to measure the ammonia nitrogen. Nitrate-nitrite nitrogen is not measured by this procedure.

For a detailed description of this procedure, see the FWQA Methods, 1970 manual

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, WPO, Cincinnati, OH 45268

AMMONIA, NITRITES AND NITRATES

I SOURCES AND SIGNIFICANCE OF AMMONIA, NITRITES AND NITRATES IN WATER

The natural occurrence of nitrogen compounds is best demonstrated by the nitrogen cycle (Figure 1).

A Ammonia

1 Occurrence

Ammonia is a product of the microbiological decay of animal and plant protein. In turn, it can be used directly to produce plant protein. Many fertilizers contain ammonia.

2 Significance

The presence of ammonia nitrogen in raw surface waters might indicate domestic pollution. Its presence in waters used for drinking purposes may require the addition of large amounts of chlorine in order to produce a free chlorine residual. The chlorine will first react with ammonia to form chloramines before it exerts its full bactericidal effects (free chlorine residual).

B Nitrites

1 Occurrence

Nitrite nitrogen occurs in water as an intermediate stage in the biological decomposition of organic nitrogen. Nitrite formers (nitrosomonas) convert ammonia under aerobic conditions to nitrites. The bacterial reduction of nitrates can also produce nitrites under anaerobic conditions. Nitrite is used as a corrosion inhibitor in industrial process water.

2 Significance

Nitrites are usually not found in surface water to a great extent.

The presence of large quantities indicates a source of wastewater pollution.

C Nitrates

1 Occurrence

Nitrate formers convert nitrites under aerobic conditions to nitrates (nitrobacter). During electrical storms, large amounts of nitrogen (N_2) are oxidized to form nitrates. Finally, nitrates can be found in fertilizers.

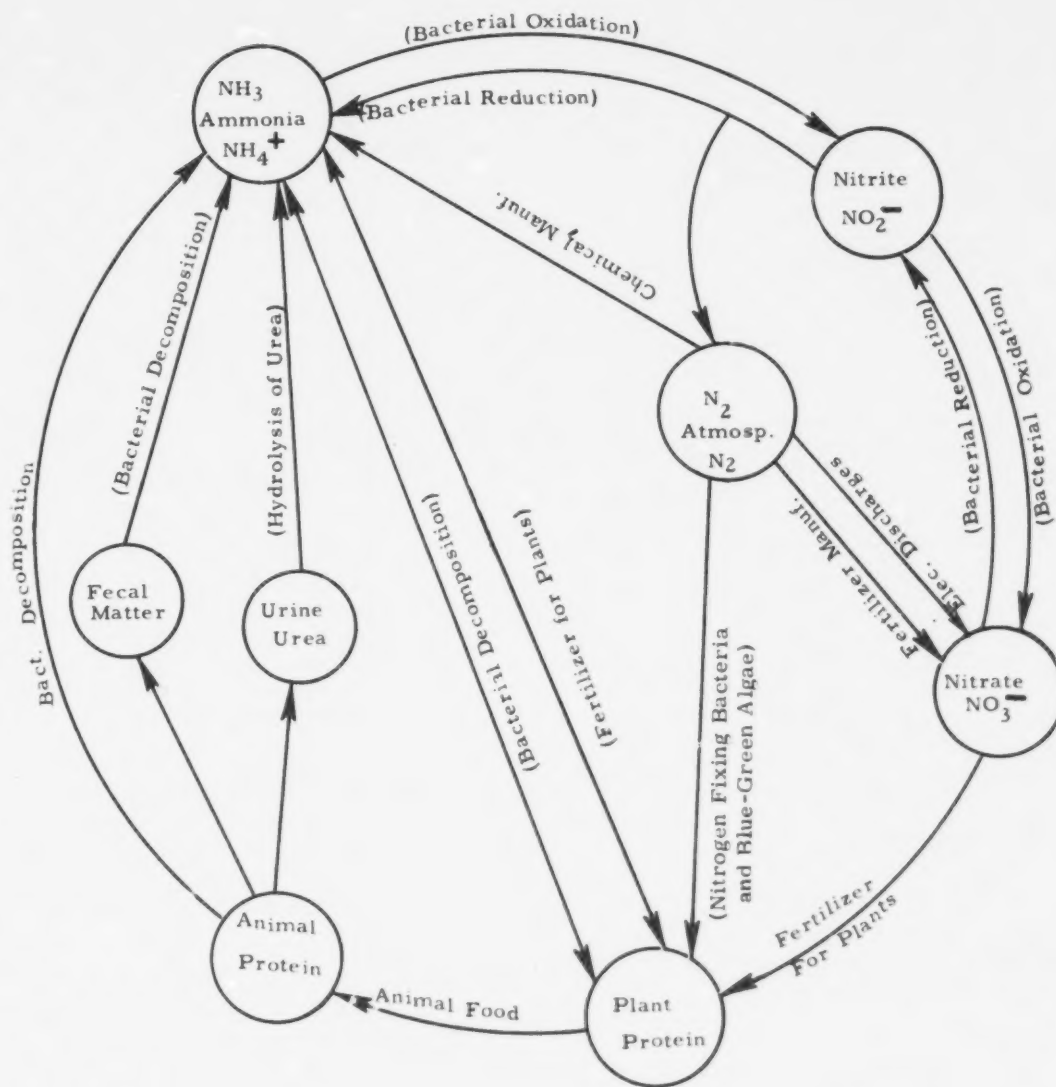
2 Significance

Nitrates in water usually indicate the final stages of biological stabilization. Nitrate rich effluents discharging into receiving waters can, under proper environmental conditions, cause stress to stream quality by producing algal blooms. Drinking water containing excessive amounts of nitrates can cause infant methemoglobinemia.

II ANALYSIS OF AMMONIA, NITRITES AND NITRATES IN WATER

A Sample Preservation⁽⁸⁾

- 1 If only ammonia is to be determined on the sample, it may be preserved with 1.0 ml of concentrated sulfuric acid per liter and stored at 4°C.
- 2 To preserve samples for any of the nitrogen procedures, add 40 mg $HgCl_2$ and store at 4°C. Even when so preserved, conversion of organic nitrogen to ammonia may occur. Preserved samples should be analyzed as soon as possible.



THE NITROGEN CYCLE

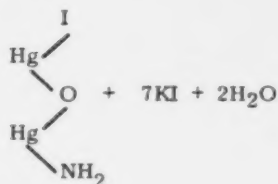
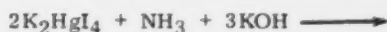
Figure 1

B Determination of Ammonia

1 Nesslerization

a Reaction

Nessler's reagent, a strong alkaline solution of potassium mercuric iodide, combines with NH_3 in alkaline solution to form a yellowish brown colloidal dispersion.



Yellow-Brown

The intensity of the color follows the Beer-Lambert Law and exhibits maximum absorption at 425 nm.

b Interferences

- 1) Nessler's reagent forms a precipitate with some ions (e.g., Ca^{++} , Mg^{++} , Fe^{++} , and S^{2-}). These ions can be eliminated in a pretreatment flocculation step with zinc sulfate and alkali. Also, EDTA or Rochelle salt solution prevents precipitation with Ca^{++} or Mg^{++} .
- 2) Residual chlorine indicates ammonia may be present in the form of chloramines. The addition of sodium thiosulfate will convert these chloramines to ammonia.
- 3) Certain organics may produce an off color with Nessler's reagent. If these compounds are not

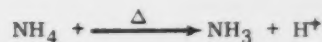
steam distillable, the interference may be eliminated in the distillation method.

- 4) If the turbidity and natural color of the sample cannot be eliminated with flocculation, it is then necessary to use the following distillation method.

2 Distillation

a Reaction

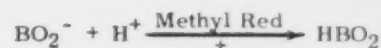
- 1) The sample is distilled in the presence of a phosphate buffer at pH 7.2 - 7.4.



Buffer

pH maintained between 7.2 - 7.4

- 2) The ammonia in the distillate is then measured by either of two techniques.
 - a) Nesslerization is used for samples containing less than 1 mg/l of ammonia nitrogen.
 - b) Absorption of NH_3 by boric acid and back titration with a standard strong acid is more suitable for samples containing more than 1 mg NH_3 N/l.



Methylene Blue

pH 7.8 - 8.0
Green

pH 6.8 - 7.0
Purple

b Interferences

- 1) Calcium reacts with the phosphate buffer releasing H^+ and lowering the pH in the distillation step. When the sample contains more than 250 mg Ca^{++}/l , it may be necessary to adjust the pH to 7.4 after adding the buffer.
- 2) Residual chlorine.
- 3) Aliphatic and aromatic amines may distill over and cause positive interference by reacting in the acid titration.

3 Precision and Accuracy

On a synthetic sample containing 0.8 mg ammonia nitrogen/l, the ARS Water Nutrients Study (1966) reported results as shown in Table 1.

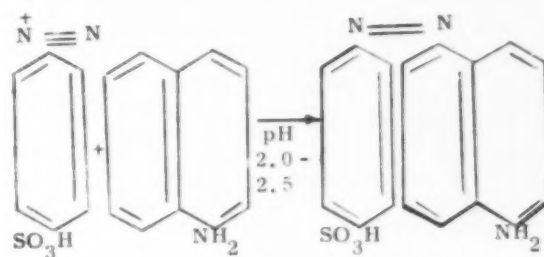
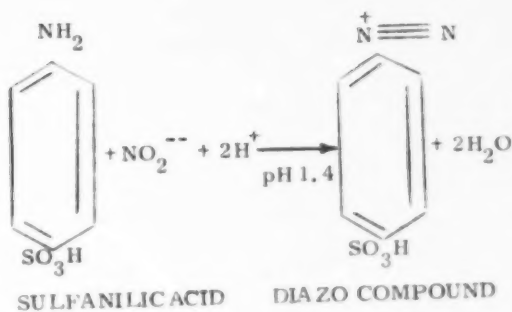
Method	Standard Deviation	Mean Error
Direct		
Nesslerization	± 0.077 mg/l	0.00 mg/l
Distillation with Nesslerization	± 0.100 mg/l	+0.02 mg/l
Distillation with Titration	± 0.167 mg/l	+0.04 mg/l

Table 1. ARS WATER NUTRIENTS, 1966

C Determination of Nitrite - Diazotization

1 Reaction

- a Under acid conditions, nitrite ions react with sulfanilic acid to form a diazo compound.
- b The diazo compound then couples with *o*-naphthylamine to form an intense red azo dye which exhibits maximum absorption at 520 nm.



2 Interferences

- a Certain ions (e.g., Hg^{++} , Ag^+ , Bi^{+++} , Sb^{+++} , Fe^{+++} , Pb^{++}) may interfere by precipitating during the test. A 0.5% EDTA solution may be used to complex iron.
- b Cupric ion introduces negative interference by catalyzing decomposition of the diazo compound.
- c Colored ions and turbidity may be removed by using $\text{Al}(\text{OH})_3$ suspension or by flocculation with zinc sulfate and alkali.

3 Precision

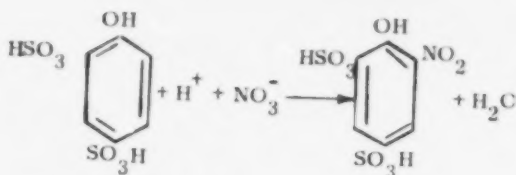
On a synthetic sample containing 0.25 mg nitrite nitrogen/l, the ARS Water Minerals Study (1961) reported 125 results with a standard deviation of ± 0.029 mg/l.

D Determination of Nitrate

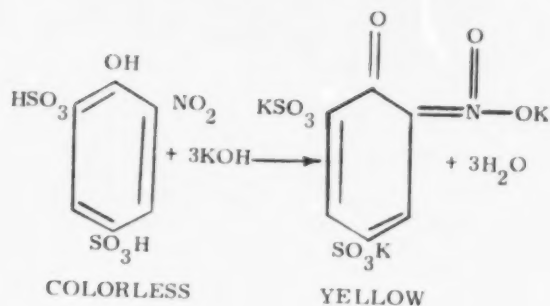
1 Phenoldisulfonic Acid

a Reaction

- 1) Phenoldisulfonic acid reacts with nitrate to produce a nitro derivative.

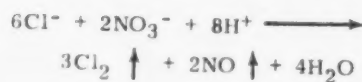


- 2) In alkaline solution the nitro derivative rearranges to form a yellow-colored compound which exhibits maximum absorption at 410 nm.



b Interferences

- 1) Chlorine ion under the acid conditions of the test introduces negative interference.



Silver sulfate can be used to precipitate Cl^- , but due to incomplete precipitation

of Ag^+ , an offcolor or turbidity is produced when the final color is developed. (Note: This difficulty can be overcome by using NH_4OH as the alkali.)

- 2) Nitrites in concentrations greater than 0.2 mg N/l introduce positive interference. However, in most waters, the concentration of nitrite is insignificant compared to nitrate.

- 3) Color and turbidity may be removed by using $\text{Al}(\text{OH})_3$ suspension or by flocculation with ZnSO_4 and alkali.

c Precision and accuracy

On a synthetic sample containing 1.0 mg nitrate N/l, and 200 mg Cl^- /l, the ARS Water Nutrients Study (1966) reported 46 results with a standard deviation of ± 0.399 mg/l and a mean error of -0.31 mg/l.

2 Brucine

a Reaction

Brucine, a strychnine compound reacts with nitrate to form a yellow compound which exhibits maximum absorption at 410 nm. The reaction according to the procedure as outlined in Standard Methods does not follow Beer's Law. However, a recent modification by Jenkins and Medsker (2) has been developed. Conditions are controlled in the reaction so that Beer's Law is followed and concentrations below 1 mg nitrate N/l can be determined.

b Interferences

- 1) Nitrite may react the same as nitrate but can be elim-

inated by the addition of sulfanilic acid to the brucine reagent

2) Organic nitrogen compounds may hydrolyze and give positive interference at low (less than 1mg/l) nitrate nitrogen concentrations.

3) Residual chlorine may be eliminated by the addition of sodium arsenite.

(8)

c Precision and Accuracy

1) Twenty-seven analysts in 15 laboratories analyzed in natural water samples containing the following increments of inorganic nitrate: 0.16, 0.19, 1.08 and 1.24 mg N/liter.

2) Precision results as standard deviation were 0.092, 0.083, 0.245, and 0.214 mg N/liter respectively.

3) Accuracy expressed as bias was -0.01, +0.02, +0.04 and +0.04 mg N/liter, respectively.

3 Hydrazine Reduction

A method using hydrazine to reduce nitrate to nitrite followed by subsequent measurement of nitrite by diazotization was recently reported by Fishman, et al. (1) The procedure has been successfully adapted to the Auto Analyzer where a high degree of control of reaction conditions can be achieved. (3)

III EPA METHODS

- A The Environmental Protection Agency, Office of Water Programs, Analytical Quality Control Laboratory, has compiled a manual of analytical methods for use by Federal laboratories. The title of this manual is "Methods for Chemical Analysis of Water & Wastes", 1971.
- B The manual lists four nitrogen constituents. These are Ammonia Nitrogen,

Total Kjeldahl Nitrogen, Nitrate Nitrogen and Nitrite Nitrogen.

1 Ammonia Nitrogen

The recommended procedures are either an automated method or distillation followed by titration (for high concentration samples) or Nesslerization (for low concentration samples)

2 Total Kjeldahl Nitrogen

The total Kjeldahl nitrogen is determined by an automated phenolate procedure, or by mercuric sulfate-sulfuric acid-potassium sulfate digestion followed by titration or colorimetric analysis

3 Nitrate Nitrogen

The automated method of determination involves reduction of the nitrate to nitrite with hydrazine, followed by colorimetric analysis. The manual method is a spectrophotometric procedure in which a yellow color is produced by the reaction the nitrate ions with brucine sulfate and sulfanilic acid

4 Nitrite Nitrogen

Nitrite is determined by diazotizing sulfanilamide with nitrite ions under acid conditions, coupling the product with N- (1-Naphthyl) ethylenediamine, and reading the resultant red-purple color at 540 nm.

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This outline was prepared by Betty Ann Punghorst, former Chemist, National Training Center and Charles Feldmann, Chemist, NTC, DTTB, MDS, WPO, EPA, Cincinnati, OH 45268.

TOTAL CARBON ANALYSIS

I INTRODUCTION

A History of Carbon Analyses

In the wake of a rapid population growth, and the increasing heavy use of our natural waterways, the nation, and indeed the world, is presented with the acute problem of increased pollutional loads on streams, rivers and other receiving bodies. This has resulted in a growing awareness of the need to prevent the pollution of streams, rivers, lakes and even the oceans. Along with this awareness has developed a desire for a more rapid and precise method of detecting and measuring pollution due to organic materials.

B The Methods

In the past, two general approaches have been used in evaluating the degree of organic water pollution.

- 1 The determination of the amount of oxygen or other oxidants required to react with organic impurities.
- 2 The determination of the amount of total carbon present in these impurities.

C Oxygen Demand Analyses

The first approach is represented by conventional laboratory tests for determining Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD). One of the principal disadvantages of these tests is that they are limited primarily to historical significance, that is, they tell what a treatment plant had been doing, since they require anywhere from two hours to five days to complete. Since up

to now no faster method has been available, traditional BOD and COD determinations have become accepted standards of measure in water pollution control work even though they are essentially ineffective for process control purposes.

Until the introduction of the Carbonaceous Analyzer, all methods taking the second approach, the total carbon method of evaluating water quality, also proved too slow.

II THE ANALYSIS OF CARBON

A Pollution Indicator

Now the carbonaceous analyzer provides a means to determine the total carbon content of a dilute water sample in approximately two minutes. With proper sample preparation to remove inorganic carbonates, the instrument determines the total organic carbon content in the sample.

B Relationship of Carbon Analysis to BOD and COD

This quantity varies with the structure from 27 percent for oxalic acid through 40 percent for glucose to 75 percent for methane. The ratio of COD to mg carbon also varies widely from 0.67 for oxalic acid through 2.67 for glucose to 5.33 for methane. Representative secondary sewage effluents have given a ratio of COD to carbon content of between 2.5 and 3.5 with the general average being 3.0.

The BOD, COD and carbon contents of these and some other representative compounds are summarized in the following table.

Total Carbon Analysis

Sample	5-Day BOD-mg/mg	COD- mg/mg	% Carbon
Stearic Acid - $C_{18}H_{36}O_2$	.786	2.91	76
Glucose - $C_6H_{12}O_6$	.73	1.07	40
Oxalic Acid - $C_2H_2O_4$	.14	.18	27
Benzoic Acid - $C_7H_6O_2$	1.38	1.97	69
Phenol - C_6H_6O	.05 to 2.1 de- pending upon concentration	2.36	77
Potassium Acid Phthalate $KHC_8H_4O_4$	.95	1.15	47
Salicylic Acid - $C_7H_6O_3$	1.25	1.60	61
Secondary Effluent, Clarified	13*	75*	21*
" " "	23*	67*	12*
" " "	4*	36*	7*

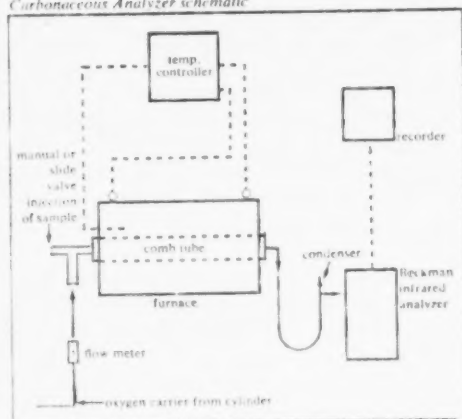
*In units of mg/l

III THE CARBON ANALYZER

A Principle of Operation

Basically the carbonaceous analyzer consists of three sections - a sampling and oxidizing system, a Beckman Model 315 Infrared Analyzer, and a strip-chart recorder.

Carbonaceous Analyzer schematic



A micro sample (20-40 μ l) of the water to be analyzed is injected into a catalytic combustion tube which is enclosed by an electric furnace thermostated at 950°C. The water is vaporized and the carbonaceous material is oxidized to carbon dioxide (CO_2) and steam in a carrier stream of pure oxygen. The oxygen flow carries the steam and CO_2 out of the furnace where the steam is condensed and the condensate removed. The CO_2 , oxygen and remaining water vapor enter an infrared analyzer sensitized to provide a measure of CO_2 . The output of the infrared analyzer is recorded on a strip chart, after which, the curve produced can be evaluated by comparing peak height with a calibration curve based upon standard solutions. Results are obtained directly in milligrams of carbon per liter.

B Application

Results (1) show that the method is applicable for most, if not all, water-soluble organic compounds -- including those that contain sulfur, nitrogen, and volatiles.

Nonvolatile organic substances can be differentiated from volatiles, such as carbon dioxide or light hydrocarbons by

determination of carbon both before and after the sample solution has been blown with an inert gas.

C Sample Preparation

The Carbonaceous Analyzer is often referred to as a total carbon analyzer because it provides a measure of all the carbonaceous material in a sample, both organic and inorganic. However, if a measure of organic carbon alone is desired, the inorganic carbon content of the sample can be removed during sample preparation.

1 Removal of inorganic carbon

The simplest procedure for removing inorganic carbon from the sample is one of acidifying and blowing. A few drops of HCl per 100 ml of sample will normally reduce pH to 2 or less, releasing all the inorganic carbon as CO_2 . Five minutes of blowing with a gas free of CO_2 sweeps out the CO_2 formed by the inorganic carbon. Only the organic carbon remains in the sample and may be analyzed without the inorganic interference.

2 Volatile carbonaceous material

Volatile carbonaceous material that may be lost by blowing is accounted for by using a dual channel carbon analyzer. Beckman's new analyzer has the previously detailed high temperature (950°C) furnace plus a low temperature (150°C) one. Using quartz chips wetted with phosphoric acid, the low temperature channel senses only the CO_2 (freed by the acid) in the original sample. The remaining organics and water are retained in the condenser connected to this low temperature furnace. None of the organics are oxidized by the 150°C furnace.

By injecting a sample into the low temperature furnace, a peak representing the inorganic carbon is obtained on the strip chart. Injecting a non-acidified sample into the high temperature furnace yields a peak representing

the total carbon. The difference between the values determined for the two peaks is the total organic carbon.

3 Dilute samples

If the sample is dilute (less than 100 mg/liter carbon) and is a true solution (no suspended particles) no further preparation is required.

4 Samples containing solids

If the sample contains solids and/or fibers which are to be included in the determination, these must be reduced in size so that they will be able to pass through the needle which has an opening of 170 microns (needles having larger openings may be obtained if necessary). In most cases, mixing the sample in a Waring Blender will reduce the particle size sufficiently for sampling.

IV PROCEDURE FOR ANALYSIS

A Interferences

Water vapor resulting from vaporization of the sample, causes a slight interference in the method. Most of the water is trapped out by the air condenser positioned immediately after the combustion furnace. However, a portion of the water vapor passes through the system into the infrared detector and appears on the strip chart as carbon. The water blank also appears on the standard calibration curve, and is therefore removed from the final calculation. In tests of solutions containing the following anions: NO_3^- , Cl^- , SO_4^{2-} , PO_4^{3-} , no interference was encountered with concentrations up to one percent.

B Precision and Accuracy

The recovery of carbon from standard solutions is 98.5 - 100.0 percent. The minimum detectable concentration using the prescribed operating instructions is 1 mg/l carbon. Generally, the data are reproducible to ± 1 mg/l with a standard deviation of 0.7 mg/l at the 100 mg/l level.

V APPLICATIONS

Several of the many research and industrial applications of the Carbonaceous Analyzer are listed below:

- A Determine the efficiency of various waste-water renovation processes, both in the laboratory and in the field.
- B Compare a plant's waste outlet with its water inlet to determine the degree of contamination contributed.
- C Monitoring a waste stream to check for product loss.
- D Follow the rate of utilization of organic nutrients by micro-organisms.
- E To detect organic impurities in inorganic compounds.

VI ADVANTAGES OF CARBON ANALYZER

A Speed

The Carbonaceous Analyzer's most important advantage is its speed of analysis. One analysis can be performed in 2-3 minutes. This speed of analysis brings about another advantage, economy of operation. Working with dilute samples, one man can run ten to twenty carbon determinations per hour. This is probably more than the number of COD or BOD tests that can even be started, much less completed, in the same period of time.

B Total Carbon

Another advantage is that the measure of carbon is a total one. The oxidizing system of the analyzer brings about complete oxidation of any form of carbon. No compound has been found to which the method is inapplicable.

VII CONCLUSIONS

The Carbonaceous Analyzer provides a rapid and precise measurement of organic carbon in both liquid and air samples. It should be found useful for many research and industrial applications, a few of which have been mentioned.

Because of its rapidity it may be found more useful than the more time-consuming BOD and COD measurements for monitoring industrial waste streams or waste treatment processes.

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This outline was prepared by Robert T. Williams, Chief, Analytical Applications Laboratory, AWTL, NERC, Cincinnati, OH 45268 and revised by Charles J. Moench, Jr., Waste Identification and Analysis, AWTL, NERC, Cincinnati, OH 45268.

DETERMINATION OF CALCIUM AND MAGNESIUM HARDNESS

I INTRODUCTION

A Definition of Hardness

USPHS - "In natural waters, hardness is a characteristic of water which represents the total concentration of just the calcium and magnesium ions expressed as calcium carbonate. If present in significant amounts, other hardness-producing metallic ions should be included."

B Other Definitions in Use

- 1 Some confusion exists in understanding the concept of hardness as a result of several definitions presently used.
- 2 Soap hardness definition includes hydrogen ion because it has the capacity to precipitate soap. Present definition excludes hydrogen ion because it is not considered metallic.
- 3 Other agencies define hardness as "the property attributable to presence of alkaline-earths".
- 4 USPHS definition is best in relation to objections of hardness in water.

II CAUSES OF HARDNESS IN WATERS OF VARIOUS REGIONS OF THE U. S.

A Hardness will vary throughout the country depending on:

- 1 Leaching action of water traversing over and through various types of geological formations.
- 2 Discharge of industrial and domestic wastes to water courses.
- 3 Uses of water which result in change in hardness, such as irrigation and water softening process.

B Objections to Hardness

- 1 Soap-destroying properties
- 2 Scale formation

C Removal and Control

Hardness may be removed and controlled through the use of various softening operations such as zeolite, lime-soda, and hot phosphate processes. It can also be removed by simple distillation or complex formation with surface active agents (detergents).

III DETERMINATION OF HARDNESS

A Two Methods in Use⁽⁶⁾

- 1 Compleximetric method (EDTA)
- 2 Calculation - by the use of appropriate factors, the hardness due to ions other than calcium and magnesium may be converted to an equivalent calcium carbonate hardness.

B Compleximetric Method

1 Principle of determination

Ethylenediaminetetra acetic acid (EDTA) is a sparingly soluble amino polycarboxylic acid which forms slightly ionized and very stable colorless complexes with the alkaline-earth metals.

- 2 Interferences: iron, manganese, nickel, and zinc.
- 3 Procedure: Time and pH considerations.
- 4 Calculations of total hardness assuming a known volume of titrant of EDTA.
- 5 Precision and accuracy.

C Determination of Calcium Hardness

1 Principle of determinations

Murexide indicator forms a salmon-colored complex with calcium whose ionization constant is of a higher value than that of the Ca EDTA complex.

2 Interferences: Heavy metals and Sr.

3 Procedure: Time of titration and proper lighting conditions are critical factors.

4 Calculation of Ca hardness.

5 Precision and accuracy.

D Determination of Magnesium Hardness

1 Calculation by difference method most commonly used.

2 Equivalent of Ca hardness is subtracted from total hardness equivalents, the difference attributable to magnesium equivalents.

3 Other methods such as pyrophosphate method, where calculation by difference method cannot be used.

IV ANALYTICAL QUALITY CONTROL METHODS

A The Environmental Protection Agency, Analytical Quality Control Laboratory has published a manual titled Methods for Chemical Analysis of Water and Wastes, 1971.

B The procedure for the determination of hardness, as discussed in this manual, was excerpted from the 13th ed. of Standard Methods⁽⁴⁾ and part 23 of ASTM, 1970⁽⁵⁾.

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- 4 Standard Methods for the Examination of Water and Wastewater, 13th ed. APHA, AWWA, WPCF, Method 122B, 1971.
- 5 ASTM Standards, Part 23, Method D1126-67, 1970.
- 6 Methods for Chemical Analysis of Water and Wastes, EPA-AQCL, Cincinnati, OH, July 1971.

This outline was prepared by B. V. Salotto, Research Chemist, Advanced Waste Treatment Branch, NERC, EPA. It was revised by C. R. Feldmann, Chemist, National Training Center, MDS, WPO, EPA, Cincinnati, OH 45268.

LABORATORY PROCEDURE FOR TOTAL HARDNESS

I REAGENTS

A Buffer Solution:

- 1 Dissolve 16.9 g of NH_4Cl in 143 ml of conc. NH_4OH .
- 2 Dissolve 1.179 g of analytical reagent grade disodium ethylenediamine tetraacetic acid dihydrate ($\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$) and 0.644 g of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ in 50 ml of distilled water.
- 3 Add the solution from (2) to the solution from (1) with mixing, and dilute to 250 ml with distilled water. Addition of small amounts of $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ or $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ may be necessary to attain exact equivalence.
- 4 The buffer should be stored in a plastic or resistant glass container tightly stoppered to prevent CO_2 absorption and NH_3 loss. Discard the buffer when 1 or 2 ml added to the sample fails to produce a pH of 10.0 ± 0.1 at the end point of the titration.

B Inhibitor:

- 1 Most water samples do not require the use of an inhibitor. In the presence of certain interfering ions, however, an inhibitor may be needed to sharpen the endpoint color change. Several types of inhibitors may be prepared or purchased. Sodium cyanide, NaCN , is one of the simpler inhibitors to use.
- 2 Add 0.25 g of powdered NaCN to the sample and adjust the pH to 9.9-10.1. Caution: NaCN is poisonous. Use large amounts of water when flushing solutions containing NaCN down the drain. Do not acidify solutions containing NaCN ; volatile, poisonous hydrogen cyanide, HCN , would be liberated.

C Indicator:

- 1 Eriochrome Black-T dye (EBT) is useful for the determination. Other commercial grades or laboratory formulations of the dye are also satisfactory.

- 2 Prepare the indicator in dry powder form by grinding together 0.5 of the dye and 100 g of NaCl .

D Standard Calcium Carbonate, CaCO_3 :

Weigh 1.000 g of anhydrous, primary standard grade CaCO_3 and transfer it to a 500 ml Erlenmeyer flask. Add 1:1 HCl (equal volumes of conc. HCl and water) dropwise and with swirling of the flask until the CaCO_3 has dissolved. Bring the volume of liquid to about 200 ml with water, boil a few seconds to dispel CO_2 , cool, and add a few drops of methyl red indicator. Adjust the color of the solution to an intermediate orange by the dropwise addition of 1:1 HCl or 1:4 NH_4OH (1 volume of conc. NH_4OH + 4 volumes of water). Transfer the solution quantitatively to a one liter volumetric flask and dilute to the mark with water. (1.0 ml = 1.0 mg CaCO_3)

E $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ (0.01 M):

Dissolve 3.723 g of the dry reagent grade $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ in distilled water and dilute to 1 liter. 1.0 ml of the 0.01 M solution = 1.0 mg of CaCO_3 . Check the concentration of this solution by titration against the standard calcium carbonate solution as described in II.

II STANDARDIZATION OF THE $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$:

- A Dilute 25.0 ml of CaCO_3 standard to about 50 ml with distilled water in a 125 ml Erlenmeyer flask against a white background.
- B Add 1-2 ml of the buffer solution and check the pH to ensure that it is 9.9 to 10.1.
- C Add approximately 0.2 g of the indicator.
- D Add the $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ slowly and with stirring until the color changes from a rose to a blue color. If the color change is not sharp, repeat the determination using the inhibitor. If the endpoint is still not sharp, prepare a fresh supply of indicator.
- E The titration should take less than 5 minutes, measured from the time of buffer addition.

Laboratory Procedure for Total Hardness

F In an analysis of this type it is advantageous to carry out a preliminary, rapid titration in order to determine approximately how much titrant will be required. This is accomplished by adding the $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ at a fast dropwise rate until the color change is observed.

III PROCEDURE

Repeat steps II A through II F using sample in place of CaCO_3 standard. The amount of sample taken should require less than 15 ml of $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ titrant.

IV CALCULATION

A Standardization of the $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$:

ml of CaCO_3 equal to 1.0 ml of the $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$

(symbol B) = $\frac{\text{ml of CaCO}_3}{\text{ml of Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O} \text{ required for titration}}$

B Total Hardness

Hardness as mg $\text{CaCO}_3/\text{l} = \frac{A \times B \times 1000}{\text{ml of sample}}$

A = ml of $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ for titration of sample

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This outline was prepared by C. R. Feldmann, Chemist, National Training Center, MDS, WPQ EPA, Cincinnati, OH 45268.

CHLORINE DETERMINATIONS AND THEIR INTERPRETATION

I INTRODUCTION

Chlorine normally is applied to water as a bactericidal agent; it reacts with water contaminants to form a variety of products containing chlorine. The difference among applied and residual chlorine represents the chlorine demand of the water under conditions specified. Wastewater chlorination is particularly difficult because the concentration of organisms and components susceptible to interaction with chlorine are high and variable. Interferences with the chlorine determination in wastewater confuse interpretation with respect to the chlorine residual at a given time and condition, its bactericidal potency, or the future behavior.

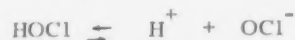
II CHEMISTRY OF CHLORINATION

A Chlorine compounds (Cl_2) dissolve in water, and hydrolyze immediately according to the reaction.



The products of this reaction are hypochlorous and hydrochloric acid. The reaction is reversible, but at pH values above 3.0 and concentrations of chlorine below 1000 mg/l the shift is predominantly to the right leading to hypochlorous acid (HOCl).

Hypochlorous acid is a weak acid and consequently ionizes in water according to the equation:



This reaction is reversible. At a pH value of 5.0 or below almost all of the chlorine is present as hypochlorous acid (HOCl) whereas above pH 10.0 nearly all of it

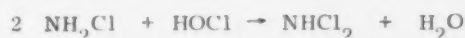
exists as hypochlorite ion (OCl^-). The pH value that will control is the pH value reached after the addition of chlorine. Chlorine addition tends to lower the pH and the addition of alkali hypochlorites tends to raise the pH.

B The initial reactions on adding chlorine to wastewaters may be assumed to be fundamentally the same as when chlorine is added to water except for the additional complications due to contaminants and their concentration.

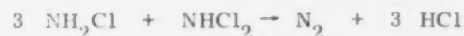
Hypochlorous acid (HOCl) reacts with ammonia and with many other complex derivatives of ammonia to produce compounds known as chloramines. Formation of the simple ammonia chloramines includes:



monochloramine



dichloramine



The distribution of the ammonia chloramines is dependent on pH, as illustrated below:

pH	Percentage of Chlorine Present as	
	Monochloramine	Dichloramine
5	16	84
6	38	62
7	65	35
8	85	15
9	94	6

Chlorine Determination and Their Interpretation

The formation of the ammonia chloramines are dependent on pH, temperature, and chlorine-ammonia ratio. Chlorine reactions with amino acids are likely; product disinfecting powers are lower than those of chlorine or of ammonia chloramines.

III TERMINOLOGY

A Terms used with Respect to Application Site

- 1 Pre-chlorination - chlorine added prior to any other treatment.
- 2 Post-chlorination - chlorine added after other treatment.
- 3 Split chlorination - chlorine added at different points in the plant - may include pre- and post-chlorination.

B Terms used in Designating Chlorine Fractions

- 1 Free available residual chlorine - the residual chlorine present as hypochlorous acid and hypochlorite ion.
- 2 Combined available residual chlorine - the residual chlorine present as chloramines and organic chlorine containing compounds.
- 3 Total available residual chlorine - the free available residual chlorine + the combined available residual chlorine - may represent total amount of chlorine residual present without regard to type.

In ordinary usage these terms are shortened to free residual chlorine, combined residual chlorine and total residual chlorine. In the chlorination of wastewaters only combined residual chlorine is ordinarily present and is often improperly termed chlorine residual.

- C Breakpoint chlorination specifically refers to the ammonia-chlorine reaction where applied chlorine hydrolyzes and reacts to form chloramines and HCl with the

chloramines eventually forming $N_2 + HCl$ as in I, B, 3. Assuming no other chlorine demand, the total chlorine residual will rise, decrease to zero and rise again with increasing increments of applied chlorine. Other substances may produce humps in the applied chlorine vs residual chlorine plot due to oxidation of materials other than ammonia. Sometimes these are erroneously considered as a breakpoint.

IV ANALYTICAL METHODS

The o-Tolidine color test and Iodometric titration methods are the basis for numerous modifications for determining chlorine residuals in water. The relative advantages of a specific determination depends upon the form in which the reactable chlorine exists and the amount and nature of interferences in the water.

Iodometric titration using the amperometric endpoint appears to be the most accurate residual chlorine method available (See current editions of Standard Methods APHA(1) and ASTM Standards (2)). The o-Tolidine and o-Tolidine Arsenite methods require little apparatus, and are readily adapted as a field or control test. The Starch Iodine color titration endpoint for iodometric titration is suitable for use on clean water or stock solutions and may be useful on certain types of wastewater residuals. Selection of a suitable method of determining chlorine residuals depends upon the correlation of the determined residual and the bacterial kill in the presence of existing interferences under applied conditions.

A Iodometric Method

1 Scope and application

This method is applicable to the determination of total chlorine residual in wastewaters, polluted waters and some industrial wastewaters.

2 Summary of method

When a sample is treated with a measured excess of standard phenylarsine oxide

solution, or a standard thiosulfate solution, followed by the addition of iodide, the iodine liberated at the proper pH is stoichiometrically proportional to the total chlorine present. The liberated iodine reacts with the phenylarsine oxide or thiosulfate before any is lost to other extraneous reactions. The excess phenylarsine oxide or thiosulfate is titrated with standard iodine solution in the presence of starch until the phenylarsine oxide or thiosulfate is completely oxidized. The end-point of the titration is the next addition of standard iodine solution that causes a faint blue color to persist in the sample.

3 Interferences

- a Organic matter - reacts with liberated iodine.
- b Manganic manganese - liberates iodine from iodide at pH 4.0.
- c Ferric iron, ferricyanide and nitrites up to 100 mg/l do not interfere at a pH of 4.0.
- d Chromates - reduce phenylarsine oxide or thiosulfate to an appreciable extent before the excess can be titrated with standard iodine.
- e Excessive color and turbidity

B Iodometric Method with Amperometric End-Point

1 Scope and application

This method is applicable to the determination of total chlorine residual in wastewaters, polluted waters and some industrial wastewaters. The back-titration method is essential for wastewaters in contrast to the direct titration with phenylarsine oxide in clean waters.

2 Summary of method

When a sample is treated with a measured excess of standard phenylarsine oxide solution followed by the addition of iodide, the iodine liberated at the proper pH is stoichiometrically proportional to the total chlorine present. The iodine liberated reacts with the phenylarsine oxide before any is lost to other extraneous reactions. When the cell is immersed in a sample so treated, no current is generated due to halogens nor is any further current generated, as the excess phenylarseneoxide is titrated with standard iodine solution until the phenylarsine oxide is completely oxidized. The end-point of the titration is the next addition of standard iodine solution that causes further current to be generated and a microammeter response or pointer deflection.

NOTE: As the end-point is approached each increment of standard iodine solution causes a temporary deflection of the microammeter, but the pointer drops back to about its original position. The true end-point is reached when a small addition of standard iodine solution gives a definite or permanent pointer deflection.

3 Interferences

- a Organic matter - reacts with the liberated iodine.
- b Manganic manganese - liberates iodine from iodide at a pH of 4.0.
- c Ferric iron, ferricyanide and nitrites up to 100 mg/l do not interfere at a pH of 4.0.
- d Chromates - reduces phenylarsine oxide to an appreciable extent before the excess can be titrated with standard iodine solution.
- e Cupric ions may cause erratic behavior of the apparatus.

Chlorine Determination and Their Interpretation

- f Cuprous and silver ions tend to poison the electrode.

C Orthotolidine Method

1 Scope and application

This method is applicable to the determination of total chlorine residual in wastewaters, polluted waters and some industrial wastewaters.

2 Summary of method

When a sample is treated with a measured amount of orthotolidine reagent, the orthotolidine is oxidized in the resulting acid solution by chlorine and chloramines and other oxidizing agents to produce a yellow-colored compound (Holoquinone). The color produced at pH values of less than 1.8 is proportional to the amount of chlorine present and is suitable for quantitative measurement. The chlorine residual in mg/l is read directly from the colored glass disks or sealed colored liquid standards or is calculated from a previously prepared standard curve. NOTE: Particular attention is called to the importance of warming the sample to 20°C after the addition of orthotolidine reagent in order to complete the reaction.

3 Interferences

- a Organic matter - oxidizes orthotolidine to produce a yellow color (Holoquinone).
- b Manganic manganese - in concentrations above 0.01 mg/l oxidizes orthotolidine to produce a yellow color (Holoquinone).
- c Ferric iron - in concentrations above 0.3 mg/l oxidizes orthotolidine to produce a yellow color (Holoquinone).
- d Nitrites - in concentrations above 0.10 mg/l of nitrite nitrogen oxidizes orthotolidine to produce a yellow color (Holoquinone).

- e Excessive color and turbidity.

D Orthotolidine- Arsenite Method

1 Scope and application

This method is applicable to the determination of free residue chlorine and combined residual chlorine in wastewaters, polluted waters and industrial wastewaters, but as normally carried out for wastewaters, etc. total residual chlorine is measured.

2 Summary of method

A sample is split into two fractions (a) and (b). Sample (a) is treated with a measured amount of arsenite reagent, followed by the addition of orthotolidine reagent. The arsenite reacts with chlorine while orthotolidine reacts with ferric iron, manganic manganese and nitrite nitrogen to produce additional color (represents interfering color). Sample (b) is treated with a measured amount of orthotolidine reagent. The orthotolidine is oxidized in the resulting acid solution by chlorine, chloramines and other oxidizing agents to produce a yellow-colored compound (Holoquinone) as described in the orthotolidine method (represents total amount of residual chlorine present and interfering color). $\text{Mg/l total residual chlorine} = b - a$ if color compensation is not made directly. NOTE: more accurate readings may be obtained if cells containing sample fractions (a) and (b) are placed in the comparator in such relative positions that color compensation is made directly.

3 Interferences

- a High color and turbidity

V INTERPRETATION

In general residual chlorine concentrations obtained by the iodometric titration method (starch iodide end-point and amperometric

end-point) will be higher than the concentrations obtained from the orthotolidine and the orthotolidine-arsenite methods. Some of the reasons for the difference have been listed under the individual methods. Extensive studies have indicated that consistently better correlation between bacterial kill and chlorine residual found is possible when the titration method is used.

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This outline was prepared by J. L. Holdaway, Chemist, Technical Program, EPA, Region III, Charlottesville, VA 22901.

THE SPECIFIC CONDUCTANCE MEASUREMENT

I INTRODUCTION

An electrical conductivity measurement of a solution determines the ability of the solution to conduct an electrical current. Very concentrated solutions have a large population of ions and transmit current easily or with small resistance. Since resistance is inversely related to conductivity $K = \frac{1}{R}$, a very concentrated solution has a very high electrical conductivity.

Electrical conductivity is determined by transmitting an electrical current through a given solution, using two electrodes. The resistance measured is dependent principally upon the ionic concentration, ionic charge, and temperature of the solution although electrode characteristics (surface area and spacing of electrodes) is also critical. Early experiments in standardizing the measurement led to construction of a "standard cell" in which the electrodes were spaced exactly 1 cm and each had a surface area of 1 cm². Using this cell, electrical conductivity is expressed as "Specific Conductance". Modern specific conductance cells do not have the same electrode dimensions as the early standard cell but have a characteristic electrode spacing/area ratio known as the "cell constant".

$$K_{sp} = \frac{1}{R} \times \frac{\text{distance (cm)}}{\text{area (cm}^2\text{)}}, \quad K_{sp} = \frac{1}{R} \times k$$

k = cell constant

Specific conductance units are Mhos/cm or reciprocal ohms/cm. Most natural, fresh waters in the United States have specific conductances ranging from 10 to 1,000 micromhos/cm. (1 micromho = 10⁻⁶ mho).

II CONDUCTIVITY INSTRUMENTS

Nearly all of the commercial specific conductance instruments are of a bridge circuit design, similar to a Wheatstone Bridge. Null or balance is detected either by meter movement, electron "ray eye" tubes, or headphones. Since resistance is directly related to temperature, some instruments have automatic temperature compensators, although inexpensive models generally have manual temperature compensation.

Conductivity instruments offer direct specific conductance readout when used with a cell "matched" to that particular instrument.

Electrodes within the cell may become damaged or dirty and accuracy may be affected; therefore, it is advisable to frequently check the instrument readings with a standard KCl solution having a known specific conductance.

III CONDUCTIVITY CELLS

Several types of conductivity cells are available, each having general applications. Dip cells are generally used for field measurement, flow cells for measurement within a closed system, and pipet cells for laboratory use. Many modifications of the above types are available for specialized laboratory applications; the Jones cells and inductive capacitance cells are perhaps the most common.

Examples of various cell ranges for the RB3 - Industrial Instruments model (0-50 micromhos/cm scale range) are in Table 1.

Cell Number	Relative Conductivity Value	Maximum range micromhos/cm	Most accurate range micromhos/cm
Cell VSO2	1	0 - 50	2 - 30
Cell VS2	10	0 - 500	20 - 300
Cell VS20	100	0 - 5000	200 - 3000

Table 1

The Specific Conductance Measurement

IV Computation of Calibration Constant

A calibration constant is a factor to which scale readings must be multiplied to compute specific conductance.

$$K_{sp} = cM$$

where K_{sp} = actual specific conductance

c = calibration constant

M = meter reading

For example, a 0.001 N KCl solution (147 micromhos/cm standard) may show a scale reading of 147.

$$147 = c \cdot 147, c = \frac{147}{147} = 1.00$$

In this case the cell is perfectly "matched" to the instrument, the calibration constant is 1.00, and the scale reading represents actual specific conductance. A variety of cells, each covering a specific range, may be used with any one instrument. However, a calibration constant for each cell must be computed before solutions of unknown specific conductance can be determined.

V RELATIONSHIP OF SPECIFIC CONDUCTANCE TO IONIC CONCENTRATION

Natural water consists of many chemical constituents, each of which may differ widely in ionic size, mobility, and solubility. Also, total constituent concentration and proportions of certain ions in various natural waters range considerably. However, it is surprising that for most natural waters having less than 2,000 mg/l. dissolved solids, specific conductance values are closely related to dissolved solids values, ranging in a ratio of .62 to .70. Of course this does not hold true for certain waters having considerable amounts of nonionized soluble materials, such as organic compounds and nonionized, colloidal inorganics.

Properties of some inorganic ions in regard to electrical conductivity are shown below:

Ion	Micromhos/cm per meq/l conc.
Calcium	52.0
Magnesium	46.6
Sodium	48.9
Potassium	72.0
Bicarbonate	43.6
Carbonate	84.6
Chloride	75.9

VI ESTIMATION OF CONSTITUENT CONCENTRATIONS

Generally speaking, for waters having a dissolved solids concentration of less than 1,000 mg/l, calcium and magnesium (total hardness), sodium, bicarbonate and carbonate (total alkalinity), and sulfate are the principal or most abundant ions, representing perhaps 90-99% of the total ionic concentration of the water. Specific conductance, total hardness and total alkalinity are all simple and expedient measurements which can be performed in the field. Therefore, the remaining principal ions are sodium and sulfate, and concentrations of these can be estimated by empirical methods. For example, we find that a certain water has:

$K_{sp} = 500$ micromhos/cm
Total Hardness = 160 mg/l or 3.20 meq/l
Total Alkalinity = 200 mg/l or 3.28 meq/l, as bicarbonate.

Next we multiply the specific conductance by *0.011 ($500 \times 0.011 = 5.50$) to estimate the total ionic concentration in meq/l.

This factor may vary slightly for different waters

Cations (meq/l)		Anions (meq/l)	
Calcium	3.20	Carbonate	0.00
Magnesium		Bicarbonate	3.28
Sodium	$5.50 - 3.20 = 2.30$	Sulfate	$5.50 - 3.28 = 2.22$
Total Cations	5.50	Total Anions	5.50

Realizing that several variables are involved in empirical analysis, application rests entirely upon testing the formula with previous complete laboratory analyses for that particular water. If correlation is within acceptable limits, analytical costs may be substantially reduced. Empirical analysis can also be used in determination of proper aliquots (dilution factor) necessary for laboratory analysis.

Records of laboratory chemical analysis may indicate that a particular stream or lake shows a characteristic response to various streamflow rates or lake water levels. If the water's environment has not been altered and water composition responds solely to natural causes, a specific conductivity measurement may be occasionally used in substitution for laboratory analyses to determine water quality. Concentration of individual constituents can thus be estimated from a specific conductance value.

VII APPLICATIONS FOR SPECIFIC CONDUCTANCE MEASUREMENTS

A Laboratory Operations⁽²⁾

- 1 Checking purity of distilled and de-ionized water
- 2 Estimation of dilution factors for samples
- 3 Quality control check on analytical accuracy
- 4 An electrical indicator

B Agriculture

- 1 Evaluating salinity
- 2 Estimating Sodium Adsorption Ratio

C Industry⁽³⁾

- 1 Estimating corrosiveness of water in steam boilers
- 2 Efficiency check of boiler operation

D Geology

- 1 Stratigraphic identification and characterization
 - a geological mapping
 - b oil explorations

E Oceanography

- 1 Mapping ocean currents
- 2 Estuary studies

F Hydrology

- 1 Locating new water supplies
 - a buried stream channels (See Fig. 1)
 - b springs in lakes and streams (See Fig. 2)
- 2 Detection and regulation of sea water encroachment on shore wells

G Water Quality Studies

- 1 Estimation of dissolved solids (See Section V, also reference 2)
- 2 Empirical analysis of constituent concentrations (See Section VI, also reference 2)
- 3 Quality control check for salt water conversion studies
- 4 Determination of mixing efficiency of streams (See Fig. 3)
- 5 Determination of flow pattern of polluted currents (See Fig. 3)
- 6 Identification of significant fluctuations in industrial wastewater effluents
- 7 Signal of significant changes in the composition of influents to waste treatment plants

FIGURE 1
DETECTION OF BURIED STREAM CHANNELS

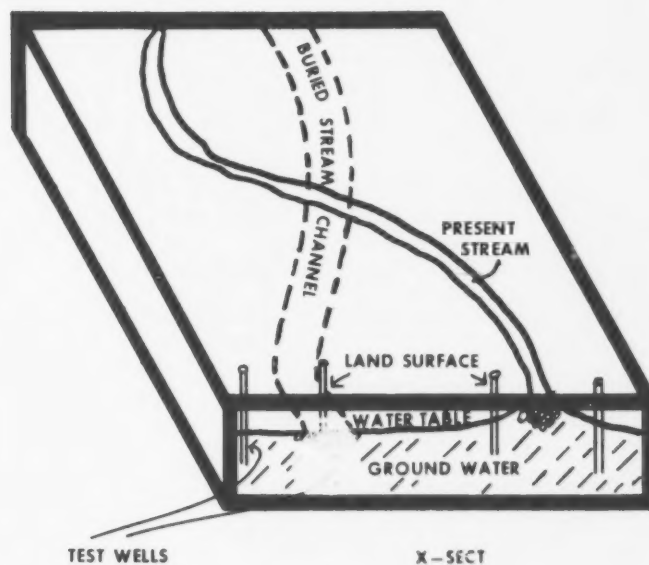
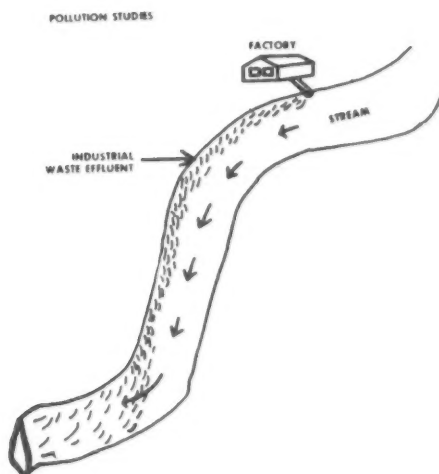


FIGURE 2
DETECTION OF SPRINGS IN LAKES AND STREAMS



FIGURE 3



VIII EPA METHODOLOGY

- A The current EPA Methods Manual⁽¹⁾ specifies that specific conductance be measured with a self-contained conductivity meter, Wheatstone bridge-type or equivalent.

Samples should preferably be analyzed at 25°C. If not, temperature corrections should be made and results reported at 25°C.

- 1 The instrument should be standardized using KCl solutions
- 2 It is essential to keep the conductivity cell clean

- B The EPA manual specifies using the procedure as described in Standard Methods⁽²⁾ or in ASTM Standards⁽³⁾.

- C Precision and Accuracy⁽¹⁾

Forty-one analysts in 17 laboratories analyzed 6 synthetic water samples containing the following K_{sp} increments of inorganic salts: 100, 106, 808, 848, 1640 and 1710 micromhos/cm.

The standard deviation of the reported values was 7.55, 8.14, 66.1, 79.6, 106 and 119 μ mhos/cm respectively.

The accuracy of the reported values was -2.0, -0.8, -29.3, -38.5, -87.9 and -86.9 μ mhos/cm bias respectively.

REFERENCES

- 1 Methods for Chemical Analysis of Water and Wastes, EPA-600/4-71-010, Cincinnati, OH 45268, 1971.
- 2 Standard Methods for the Examination of Water and Wastewater, APHA-AWWA-WPCF, 13th edition, 1971.
- 3 ASTM Standards, Part 23, 1970.

This outline was prepared by John R. Tilstra, Chemist, National Eutrophication Research Program, Corvallis, Oregon with additions by Audrey E. Donahue, Chemist, DTTB, MDS, WPO, EPA, Cincinnati, OH 45268.

DETERMINATION OF PHOSPHORUS IN THE AQUEOUS ENVIRONMENT

I Phosphorus is closely associated with water quality because of (a) its role in aquatic productivity such as algal blooms, (b) its sequestering action, which causes interference in coagulation, (c) the difficulty of removing phosphorus from water to some desirable low concentration, and (d) its characteristic of converting from one to another of many possible forms.

A Phosphorus is one of the primary nutrients such as hydrogen (H), carbon (C), nitrogen (N), sulfur (S) and phosphorus (P).

1 Phosphorus is unique among nutrients in that its oxidation does not contribute significant energy because it commonly exists in oxidized form.

2 Phosphorus is intimately involved in oxidative energy release from and synthesis of other nutrients into cell mass via:

a Transport of nutrients across membranes into cell protoplasm is likely to include phosphorylation.

b The release of energy for metabolic purposes is likely to include a triphosphate exchange mechanism.

B Most natural waters contain relatively low levels of P (0.01 to 0.05 mg/l) in the soluble state during periods of significant productivity.

1 Metabolic activity tends to convert soluble P into cell mass (organic P) as a part of the protoplasm, intermediate products, or sorbed material.

2 Degradation of cell mass and incidental P compounds results in a feedback of lysed P to the water at rates governed by the type of P and the environment. Aquatic metabolic kinetics show marked influences of this feedback.

3 The concentrations of P in hydrosols, sludges, treatment plant samples and soils may range from 10^2 to 10^6 times that in stabilized surface water. Both concentration and interfering components affect applicability of analytical techniques.

II The primary source of phosphorus in the aqueous system is of geological origin. Indirect sources are the processed mineral products for use in agriculture, household, industry or other activities.

A Agricultural fertilizer run-off is related to chemicals applied, farming practice and soil exchange capacity.

B Wastewaters primarily of domestic origin contain major amounts of P from:

1 Human, animal and plant residues

2 Surfactants (cleaning agent) discharge

3 Microbial and other cell masses

C Wastewaters primarily of industrial origin contain P related to:

1 Corrosion control

2 Scale control additives

3 Surfactants or dispersants

4 Chemical processing of materials including P

5 Liquors from clean-up operations of dusts, fumes, stack gases, or other discharges

III Phosphorus terminology is commonly confused because of the interrelations among biological, chemical, engineering, physical, and analytical factors.

Determination of Phosphorus in the Aqueous Environment

- A Biologically, phosphorus may be available as a nutrient, synthesized into living mass, stored in living or dead cells, agglomerates, or mineral complexes, or converted to degraded materials.
- B Chemically, P exists in several mineral and organic forms that may be converted from one to another under favorable conditions. Analytical estimates are based upon physical or chemical techniques necessary to convert various forms of P into ortho phosphates which alone can be quantitated in terms of the molybdenum blue colorimetric test.
- C Engineering interest in phosphorus is related to the prediction, treatment, or control of aqueous systems to favor acceptable water quality objectives. Phosphorus removal is associated with solids removal.
- D Solubility and temperature are major physical factors in phosphorus behavior. Soluble P is much more available than insoluble P for chemical or biological transformations and the rate of conversion from one to another is strongly influenced by temperature.
- E Table 1 includes a classification of the four main types of chemical P and some of the relationships controlling solubility of each group. It is apparent that no clear-cut separation can be made on a solubility basis as molecular weight, substituent and other factors affect solubility.
- F Table 2 includes a scheme of analytical differentiation of various forms of P based upon:
 - 1 The technique required to convert an unknown variety of phosphorus into ortho P which is the only one quantitated by the colorimetric test.
- 2 Solubility characteristics of the sample P or more precisely the means required to clarify the sample.
 - a Any clarification method is subject to incomplete separation. Therefore, it is essential to specify the method used to interpret the yield factor of the separation technique. The degree of separation of solubles and insolubles will be significantly different for:
 - 1 Membrane filter separation (0.5 micron pore size)
 - 2 Centrifugation (at some specified rpm and time)
 - 3 Paper filtration (specify paper identification)
 - 4 Subsidence (specify time and conditions)
- G Analytical separations (Table 2) like those in Table 1, do not give a precise separation of the various forms of P which may be included quantitatively with ortho or poly P. Conversely some of the poly and organic P will be included with ortho P if they have been partially hydrolyzed during storage or analysis. Insolubles may likewise be included as a result of poor separation and analytical conditions.
 - 1 The separation methods provide an operational type of definition adequate in most situations if the "operation" is known. Table 2 indicates the nature of incidental P that may appear along with the type sought.

Table 1

PHOSPHORUS COMPOUNDS CLASSIFIED BY
CHEMICAL AND SOLUBILITY RELATIONS

Form	Water Soluble ⁽¹⁾	Insoluble ⁽¹⁾
1. Ortho phosphates $(\text{PO}_4)^{-3}$	Combined with monovalent cations such as H, Na, K, NH_4	Combined with multivalent cations such as Ca^{+2} , Al^{+3} , Fe^{+3}
2. Poly phosphates $(\text{P}_2\text{O}_7)^{-4}$, $(\text{P}_3\text{O}_{10})^{-5}$, $(\text{P}_3\text{O}_9)^{-3}$ and others depending upon the degree of dehydration.	as in 1 above Increasing dehydration decreases solubility	(a) as in 1 above (b) multi P polyphosphates (high mol. wt.) including the "glassy" phosphates
3. Organic phosphorus R-P, R-P-R (2) (unusually varied nature)	(a) certain chemicals (b) degradation products (c) enzyme P (d) phosphorylated nutrients	(a) certain chemicals (b) cell mass, may be colloidal or agglomerated (c) soluble P sorbed by insoluble residues
4. Mineral phosphorus	(a) as in 1 above	(a) as in 1 above (b) as in 2 above (c) geological P such as phosphosilicates (d) certain mineral complexes.

(1) Used in reference to predominance under common conditions.

(2) R represents an organic radical, P represents P , PO_4^{-3} , or its derivatives.

2 Total P in Table 2 includes liquid and separated residue P that may exist in the whole sample including silt, organic sludge, or hydrosols. This recognizes

that the feedback of soluble P from deposited or suspended material has a real effect upon the kinetics of the aqueous environment.

Table 2

**PHOSPHORUS COMPOUNDS CLASSIFIED BY
ANALYTICAL METHODOLOGY**

Desired P Components	Technique ⁽¹⁾	Incidental P Included ⁽²⁾
1. Ortho phosphates	No treatment on clear samples	Easily hydrolyzed (a) poly phosphates - (b) organic -P, - (c) Mineral -P, + or -
2. Polyphosphates (2)-(1) = poly P (hydrolyzable)	acid hydrolysis on clear samples, dilute (a) H_2SO_4 (b) HCl heated	(a) ortho-P + (b) organic -P + or - (c) mineral -P + or -
3. Organic phosphorus (3) - (2) + org P (hydrolyzable)	acid + oxidizing hydrolysis on whole sample, dilute (a) $H_2SO_4 + HNO_3$ (b) $H_2SO_4 + (NH_4)_2S_2O_8$ heated	(a) ortho P + (b) poly P + (c) mineral P + or -
4. Soluble phosphorus (preferably classified by clarification method)	clarified liquid following filtration, centrifugation or subsidence	generally includes (a) 1, 2, or 3 (b) particulates not completely separated
5. Insoluble phosphorus (residue from clarification)	Retained residues separated during clarification See (6)	(a) generally includes sorbed or complexed solubles.
6. Total phosphorus	Strong acid + oxidant digestion (a) $H_2SO_4 + HNO_3$ (b) $H_2SO_4 + HNO_3 + HClO_4$ (c) $H_2O_2 + Mg(NO_3)_2$ fusion	all components in 1, 2, 3, 4, 5 in the whole sample

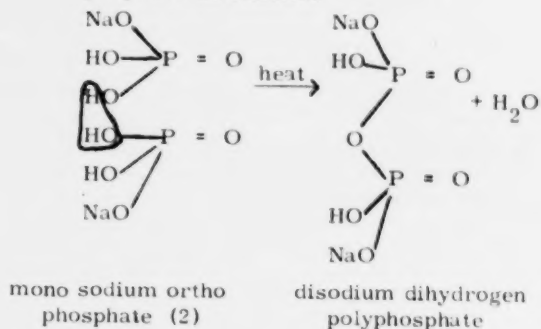
(1) Determinative step by phospho molybdate colorimetric method.

(2) Coding: + quantitative yield
- a small fraction of the amount present
+ or - depends upon the individual chemical and sample history

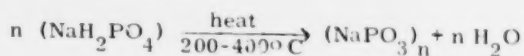
IV Polyphosphates are of major interest in cleaning agent formulation, as dispersants, and for corrosion control.

A They are prepared by dehydration of ortho phosphates to form products having two or more phosphate derivatives per molecule.

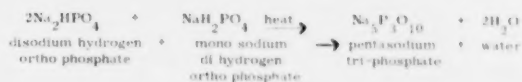
1 The simplest polyphosphate may be prepared as follows:



2 The general form for producing polyphosphates from mono substituted orthophosphates is:



3 Di-substituted ortho phosphates or mixtures of substituted ortho phosphates lead to other polyphosphates:



4 The polyphosphate series usually consist of the polyphosphate anion with a negative charge of 2 to 5. Hydrogen or metals commonly occupy these sites. The polyphosphate can be further dehydrated by heat as long as hydrogen remains. Di or trivalent cations generally produce a more

insoluble polyphosphate than the same cation in the form of insoluble ortho phosphate. Insolubility increases with the number of P atoms in the polyphosphate. The "glassy" polyphosphates are a special group with limited solubility that are used to aid corrosion resistance in pipe distribution systems and similar uses.

B Polyphosphates tend to hydrolyze or "revert" to the ortho P form by addition of water. This occurs whenever polyphosphates are found in the aqueous environment.

1 The major factors affecting the rate of reversion of poly to orthophosphates include:

- a) Temperature, increased T increases rate
- b) pH, lower pH increases rate
- c) Enzymes, hydrolase enzymes increase rate
- d) Colloidal gels, increase rate
- e) Complexing cations and ionic concentration increase rate
- f) Concentration of the polyphosphate increases rate

- 2 Items a, b and c have a large effect upon reversion rate compared with other factors listed. The actual reversion rate is a combination of listed items and other conditions or characteristics.
- 3 The differences among ortho and ortho + polyphosphates commonly are close to experimental error of the colorimetric test in stabilized surface water samples. A significant difference generally indicates that the sample was obtained relatively close to a source of polyphosphates and was promptly analyzed. This implies that the reversion rate of polyphosphates is much higher than generally believed.

V SAMPLING AND PRESERVATION TECHNIQUES

A Sampling

- 1 Great care should be exercised to exclude any benthic deposits from water samples.
- 2 Glass containers should be acid rinsed before use.
- 3 Certain plastic containers may be used. Possible adsorption of low concentrations of phosphorus should be checked.
- 4 If a differentiation of phosphorus forms is to be made, filtration should be carried out immediately upon sample collection. A membrane filter of 0.45μ pore size is recommended for reproducible separations.

B Preservation

- 1 If at all possible, samples should be analyzed on the day of collection. At best, preservation measures only retard possible changes in the sample.
 - a Possible physical changes include solubilization, precipitation, absorption on or desorption from suspended matter.
 - b Possible chemical changes include reversion of poly to ortho P and decomposition of organic or mineral P.
 - c Possible biological changes include microbial decomposition of organic P and algal or bacterial growth forming organic P.
- 2 Refrigeration at 4°C is recommended if samples are to be stored. This decreases hydrolysis and reaction rates and also losses due to volatility.

- 3 Addition of $40\text{ mg HgCl}_2/\text{liter}$ is recommended for longer storage periods. This chemical limits biological changes.

- a HgCl_2 is an interference in the analytical procedure if the chloride level is low (See Part VI, B3).

VI THE EPA ANALYTICAL PROCEDURE⁽⁶⁾

- A This is a colorimetric determination, specific for orthophosphate. Depending on the nature of the sample and on the type of data sought, the procedure involves two general operations:

- 1 Conversion of phosphorus forms to soluble orthophosphate (See Fig. 1):

- a sulfuric acid-hydrolysis for polyphosphates, and some organic P compounds.
- b persulfate digestion for organic P compounds.

- 2 The color determination involves reacting dilute solutions of phosphorus with ammonium molybdate and potassium antimonyl tartrate in an acid medium to form an antimony-phosphomolybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the orthophosphate concentration.

Color absorbance is measured at 880 nm and a concentration value obtained using a standard curve.

Reagent preparation and the detailed procedure can be found in the EPA manual.

The methods described there are usable in the 0.01 to 0.5 mg/liter phosphorus range.

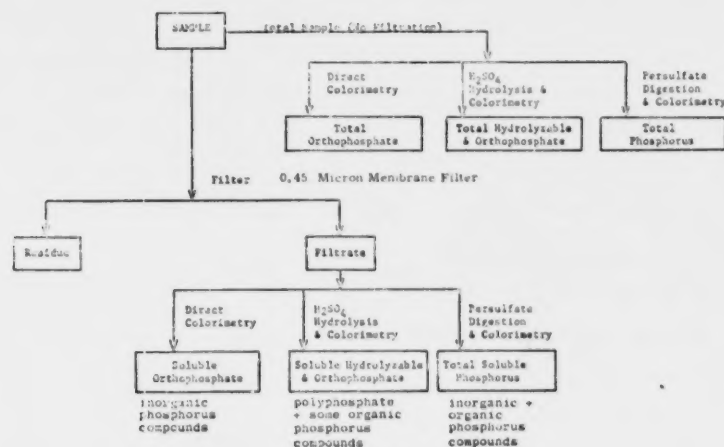


FIGURE 1
ANALYTICAL SCHEME FOR DIFFERENTIATION OF PHOSPHORUS FORMS

B Interferences

- 1 Erroneous results from contaminated glassware is avoided by cleaning it with hot 1:1 HCl, treating it with procedure reagents and rinsings with distilled water. Preferably this glassware should be used only for the determination of phosphorus and protected from dust during storage. Commercial detergents should never be used.
- 2 High iron concentrations in samples can precipitate phosphorus.
- 3 If HgCl_2 is used as a preservative, it interferes if the chloride level of the sample is less than 50 mg Cl/liter. Spiking with NaCl is then recommended.
- 4 Others have reported interference from chlorine, chromium, sulfides, nitrite, tannins, lignin and other minerals and organics at high concentrations.

C Precision and Accuracy⁽⁶⁾

- 1 Organic phosphate - 33 analysts in 19 laboratories analyzed natural water samples containing exact increments of organic phosphate of 0.110, 0.132, 0.772, and 0.882 mg P/liter.

Standard deviations obtained were 0.033, 0.051, 0.130 and 0.128 respectively.

Accuracy results as bias, mg P/liter were: +0.003, +0.016, +0.023 and - 0.008, respectively.

- 2 Orthophosphate was determined by 26 analysts in 16 laboratories using samples containing orthophosphate in amounts of 0.029, 0.038, 0.335 and 0.383 mg P/liter.

Standard deviations obtained were 0.010, 0.008, 0.018 and 0.023 respectively.

Accuracy results as bias, mg P/liter were -0.001, -0.002, -0.009 and -0.007 respectively.

VII VARIABLES IN THE COLORIMETRIC PROCEDURE

Several important variables affect formation of the yellow heteropoly acid and its reduced form, molybdenum blue, in the colorimetric test for P.

A Acid Concentration during color development is critical. Figure 2 shows that color will appear in a sample containing no phosphate if the acid concentration is low. Interfering color is negligible when the normality with respect to H_2SO_4 approaches 0.4.

- 1 Acid normality during color development of 0.3 to slightly more than 0.4 is feasible for use. It is preferable to control acidity carefully and to seek a normality closer to the higher limits of the acceptable range.
- 2 It is essential to add the acid and molybdate as one solution.
- 3 The aliquot of sample must be neutralized prior to adding the color reagent.

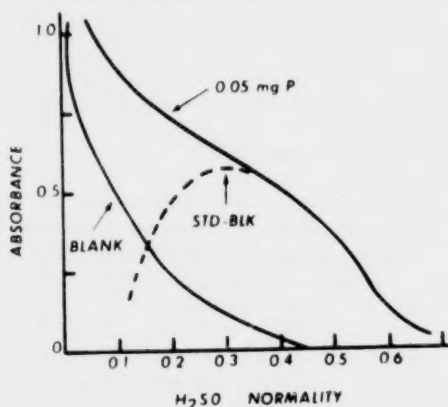


Figure 2

0-PHOSPHATE COLOR
VS ACIDITY

B Choice of Reductant - Reagent stability, effective reduction and freedom from deleterious side effects are the bases for reductant selection. Several reductants have been used effectively. Ascorbic acid reduction is highly effective in both marine and fresh water. It is the reductant specified in the EPA method.

C Temperature affects the rate of color formation. Blank, standards, and samples must be adjusted to the same temperature ($\pm 1^\circ C$), (preferably room temperature), before addition of the acid molybdate reagent.

D Time for Color Development must be specified and consistent. After addition of reductant, the blue color develops rapidly for 10 minutes then fades gradually after 12 minutes.

VIII DETERMINATION OF TOTAL PHOSPHORUS

- A Determination of total phosphorus content involves omission of any filtration procedure and using the acid-hydrolysis and persulfate treatments to convert all phosphorus forms to the test-sensitive orthophosphate form.
- B Determining total phosphorus content yields the most meaningful data since the various forms of phosphorus may change from one form to another in a short period of time. (See part V, B1)

IX DEVELOPMENT OF A STANDARD PROCEDURE

Phosphorus analysis received intensive investigation; coordination and validation of methods is more difficult than changing technique.

A Part of the problem in methods arose because of changes in analytical objectives such as:

- 1 Methods suitable to gather "survey" information may not be adequate for "standards".
- 2 Methods acceptable for water are not necessarily effective in the presence of significant mineral and organic interference characteristic of hydrosoils, marine samples, organic sludges and benthic deposits.
- 3 Interest has been centered on "fresh" water, it was essential to extend them for marine waters.

4 Instrumentation and automation have required adaptation of methodology.

B Analysts have tended to work on their own special problems. If the method apparently served their situations, it was used. Each has a "favorite" scheme that may be quite effective but progress toward widespread application of "one" method has been slow. Consequently, many methods are available. Reagent acidity, Mo content, reductant and separation techniques are the major variables.

C At the present time there is not sufficient data to warrant EPA endorsement of the P procedure for sediment-type samples, sludges, algal blooms, etc. Following is a procedure (not included in the EPA manual) which is useful when solids are present in samples:

- 1 If sample contains large particles, grind and emulsify solids in a blender.
- 2 Transfer 50 ml sample, or aliquot diluted to 50 ml, into a 250 ml Erlenmeyer flask.
- 3 Add 6.0 ml of 18N H_2SO_4 , 5 ml concentrated HNO_3 , 2 berl saddles and digest on hot plate.

- 4 Digest until the disappearance of nitric acid fumes and the appearance of white SO_3 fumes. Continue digestion for approximately 5 minutes. Cool before proceeding with Step 5.
- 5 Add 2 ml of HNO_3-HClO_4 mixture and 5 ml concentrated HNO_3 . Continue digestion until all of the nitric acid is driven off and dense fumes of perchloric evolve. Perchloric acid requires dilution with sulfuric acid and prior destruction of most organics for safety.
- 6 Cool. Add approximately 40 ml distilled water and transfer to 100 ml volumetric flask.
- 7 Add 2-3 drops of phenolphthalein and concentrated ammonium hydroxide until a pink color is seen. Then discharge the pink color with the strong sulfuric acid. It is advisable to add an equivalent amount of salt formed during neutralization of digested samples to the calibration standards to equalize salt content during color development.
- 8 Determine orthophosphate according to the usual color procedure.

ACKNOWLEDGMENT:

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This outline was prepared by Audrey E. Donahue, Chemist, National Training Center, DTTB, MDS, WPQ, EPA, Cincinnati, OH 45268.

SOURCES, EFFECTS AND ANALYSES OF CYANIDES

I SOURCES, EFFECTS AND ANALYSES OF CYANIDES

- A Cyanides, generally, are the first consideration in fish kills, just as phenols are commonly credited for objectionable water tastes or odors. The fact that many other pollutants or conditions can and do cause similar effects may not prevent unjust accusations. Care must be used in discharge and in the records to protect the public and the processor.
- B Fish toxicity has been reported at 0.025 mg CN⁻/liter. Microorganisms generally are more tolerant. Certain organisms are adaptable to the destruction of cyanides. Activated sludge or trickling filters successfully destroyed 60 mg/l of feed cyanide, producing a nitrified effluent with negligible cyanide. Cyanide is an enzyme blocking agent; therefore, toxicity depends upon reactivity of essential enzymes and cyanide and the relative fraction of inactivated enzyme. HCN is similar in action to CO in human toxicity.

II INDUSTRIAL SOURCES OF CYANIDES

- A Metal Plating
- B Case Hardening
- C Metal Cleaning Baths
- D Silver and Gold Refining
- E Gas Scrubbers from Pyrolytic Processes (Coking, Refining, Blast Furnaces)
- F Rubber Acrylic Fiber, Plastic Industry (Mainly as Copolymers with Nitriles)
- G Chemical Process Intermediates

III CYANIDE TREATMENT

- A Lagooning
May lead to complexation, oxidation, polymerization, or volatilization.
- B Acidification and Aeration (Transfers HCN to the Air)
- C Lime-Sulfur Treatment (Forms Less Toxic SCN⁻)
- D Complexation with Heavy Metals (Of Doubtful Value)
- E Precipitation as Heavy Metal Salts (Requires Close Control)
- F Oxidation with KMnO₄, ClO₂, O₃, Cl₂
Alkaline chlorination currently gives best results. Cr⁺⁶ oxidation conditions have not been found.
- G Biochemical Oxidation (For Low Concentrations)
- H Ion Exchange
- I Electrolytic Oxidation (For High Concentrations)

IV Reactivity and variety in the form of cyanides cause major difficulties in treatment or analysis, and each problem requires individual handling.

- A Major Forms of Cyanide
- 1 Simple soluble cyanides - H⁺, Na⁺, K⁺, NH₄⁺.
 - 2 Simple insoluble cyanides - Ni(CN)₂, Zn⁺², Cd⁺², etc.
 - 3 Complexed cyanides - association products of 1 and 2.

- 4 Organic Cyanides - R-CN (Nitriles)
 - 5 Cyanates (OCN⁻) and Thiocyanates (SCN⁻)
 - 6 Organic compounds degrading to form HCN during analysis (glycine).
- B Item A1 is the only one that can be treated or analyzed efficiently. Other forms must be converted to simple soluble cyanides during or prior to treatment or analysis. Other forms of cyanide may be considered as interferences.
- V ANALYTICAL INTERFERENCE CONTROL
- A Insoluble and complexed cyanides generally can be converted to simple cyanides by the Serfass distillation technique in the presence of HgCl₂, MgCl₂, and acid.
- $$\text{K}_2\text{Zn}(\text{CN})_4 + 2\text{HgCl}_2 \longrightarrow 2\text{Hg}(\text{CN})_2 + 2\text{KCl} + \text{ZnCl}_2 \quad (1)$$
- $$\text{Hg}(\text{CN})_2 \xrightarrow{\text{Cl}^-} \text{Hg}^{++} + 2\text{CN}^- \quad (2)$$
- $$2\text{CN}^- + \text{H}^+ \rightleftharpoons \text{HCN} \quad (3)$$
- Certain copper and cobalt complexes and probably others are not converted completely to simple cyanides except after extended distillation periods.
- B Sulfides - Precipitate with PbCO₃ before analysis.
- C Fatty Acids or Other Organic Turbidity
Frequently extracted with iso-octane, hexane, or chloroform, from slightly acid solution.
- D Amines
Extract from slightly alkaline solution as in V C. The three solvents will not extract detectable HCN under specified conditions.
- E Oxidizing agents may destroy HCN. Test with starch-KI paper and titrate with sodium sulfite to a negative test before analysis.
- F Turbidity - Remove by acid distillation. Trace quantities may be controlled by extraction of the cyanide color complex.
- G Color - Try extraction, distillation, or both.
- H SCN⁻ and OCN⁻ (Acid distillation destroys them)
- I Organic cyanides or organics (apparently forming cyanide under analytical conditions) generally interfere to a degree depending upon the individual constituent. No general method of removal can be recommended. Results are open to question, as these constituents may produce false cyanide tests. Nitriles and organic nitrogen compounds may produce low level interference. Acid distillation may help.
- J Buffer capacity of the sample may raise the pH above 8.0 during color formation, which decreases resulting absorbance materially in the pyridine parazolone test.
- K The CN⁻ group is one of the most highly reactive organic functional groups in organic chemistry. Simple, easily hydrolyzed cyanide compounds rarely persist in water for more than a few hours unless preserved or combined with inorganic or organic components that stabilize the system.
- 1 Interference control involves complex operations favoring cyanide losses. The separation of interfering components and cyanide rarely are complete. These factors invariably present a compromise situation among interference control techniques favoring complete separation from spurious high or confusing results or losses of cyanide as a result of time and manipulation.

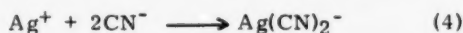
- 2 Cyanide results must be interpreted in line with sample history and manipulation. Apparent cyanide results of approximately 0.01 mg CN^-/l may be due to interference components in the sample. High analytical recovery is unlikely for stable compounds or complexes of cyanide.

VI ESTIMATION OF CYANIDE

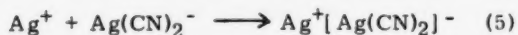
Three methods are commonly used for the quantitative estimation of cyanide after prior treatment to remove interferences, if necessary. These include: titration with silver nitrate preferred for samples containing more than 1 mg CN^-/l ; benzidine-pyridine color method for clear samples of stabilized water; and the pyridine pyrazolone color technique for samples containing higher organic interference.

Both color tests are applicable for low concentrations of cyanide, with preference depending upon the nature of the sample.

- A The silver nitrate titration includes the titration of cyanide in an alkaline sample ($\text{pH} < 11$) with a standard silver nitrate solution in the presence of p-dimethylaminobenzal rhodanine indicator.



- 1 As long as there is an excess of cyanide in the sample titrated, the silver will combine as the anion $\text{Ag}(\text{CN})_2^-$, which will not cause the indicator color change.
- 2 When all of the cyanide has been combined as the product of (4), an excess of AgNO_3 leads to an excess of Ag^+ , as follows:



- 3 The rhodanine indicator exhibits a canary yellow color in the presence of the $\text{Ag}(\text{CN})_2^-$ anion. It changes to a salmon hue with the first excess of Ag^+ cation, as in (5).

- 4 This indicator is stable under acid conditions and is normally so used for heavy metal cation detection. Under the alkaline conditions for this titration, the indicator is less stable and may change in color with time. If titration requires more than five minutes time under alkaline conditions, it is advisable to add fresh indicator to improve the sharpness of the color change or to repeat the titration until the color change point is approached.

- 5 The color change is confusing for the apprentice, but becomes more distinct with experience, provided titration time is no longer than five minutes.

- B The benzidine-pyridine cyanide test involves formation of an addition product of cyanide by bromination. The cyanogen bromide reacts with benzidine in the presence of pyridine to form an orange to red color compound with maximum absorbance at 480 nm.

- 1 The test is simple to perform on clear samples that do not have an appreciable bromine demand other than that for cyanide.
- 2 Turbid or colored samples require clarification by distillation, or otherwise, to enable the analyst to discern when there is an excess of bromine color and to control the addition of sodium arsenite to remove excess bromine.
- 3 Stabilized waters commonly have a bromine demand low enough for application of this test. When the sample has a high and variable bromine demand due to unstable organic residues, Method C may be more applicable.

- C The pyridine pyrazolone color test is more applicable for unstable samples such as industrial wastewaters where high bromine demands may be encountered.

Certain of the reagents are unstable and technique control is more involved than with Method B. More frequent rechecks of reagents and calibrations are necessary for valid results, but with test experience the analyst can use the test effectively on a wide variety of samples.

- 1 The test, as described on page 455 of Standard Methods, includes halogenation of the sample with Chloramine T and the addition of a mixed reagent containing 3,3'-dimethyl-1,1'-diphenyl-4,4'-bi-2 pyrazoline)-5,5'-dione (Eastman 6969) in a saturated solution of 1-phenyl-3-methyl-5-pyrazoline.
- 2 Maximum color absorbance is 620 nm in the aqueous color mix. The color may be extracted with n-butyl alcohol after color formation in the aqueous phase. Extraction produces a slight color shift with a maxima at 630 mμ.
- 3 Chloramine T halogenates the sample cyanide effectively with a standard addition volume, but apparently does not react with as many other sample materials contributing to high bromine demands for Method B. The Chloramine T is unstable. If the Chloramine T does not dissolve promptly as a 1% aqueous solution, the analyst should acquire a fresh supply of high quality reagent.
- 4 The individual components of the mixed reagent are relatively stable. Once mixed, it should be used within a few hours because the blank color increases while the color per increment of cyanide decreases with time.

VII GENERAL PRECAUTIONS FOR CYANIDE DETERMINATIONS

- A Available methods cannot completely separate "free" and "total" cyanides.
- 1 "Free" cyanide is considered to be that cyanide combined with H^+ , Na^+ , K^+ , or NH_4^+ form in the sample or converted to that form during analysis.

"Total" cyanide is considered to be the sum of free cyanide plus complexed or combined cyanides converted to the free cyanide from by specified analytical routines prior to the titration or color tests.
 - 2 The materials measured as free cyanide, or readily converted to that form, are considered most significant from a toxicity standpoint.
 - 3 Total cyanide is of interest because light, pH, and associated factors may shift and cause a breakdown of materials containing cyanides not indicated by direct cyanide determination.
 - 4 Many complex cyanide association products may respond during direct analysis as free cyanide, or indicate erroneously high values due to interference. Other complexes yield small fractions of cyanide upon direct analysis compared to the total amount present.
 - 5 The primary considerations include the amount of cyanide existing as HCN, or other simple cyanides in the system at a given time, and the ease with which the existing equilibrium may be shifted by conditions to favor the release of more reactive forms of cyanide from the stable complexes.

B Serfass distillation is recommended whenever the sample is anticipated to contain significant concentrations of heavy metals. Distillation is useful for removal of turbidity and many other interfering components.

- 1 The importance of distillation can not be over-emphasized. It should be part of the analytical routine unless trials with and without distillation provide results comparable within the limits of experimental errors.
- 2 Either HgCl_2 or Cu_2Cl_2 under acid conditions provide effective breakdown and release of recoverable cyanide during distillation of most complexes. The distillation appears to provide higher yields of recoverable cyanide in the presence of a high chloride concentration.
- 3 The Serfass reflux technique preferably should be split into two 1-hour reflux periods. The first 1-hour period will recover easily hydrolyzed cyanides. Yield of cyanide during the second hour after cooling the distillation mixture and restarting the routine with a fresh absorbent charge suggests form and yield of sample cyanides.

Negligible cyanide recovery during the second hour indicates high analytical recovery from a sample containing readily released cyanides. A significant cyanide yield during the second hour indicates presence of relatively stable cyanides and probably a low analytical recovery. Two reflux periods are not advisable when the sample contains low cyanide levels.

C Sample preservation is never 100% effective. Complexes may form, rearrange, or dissociate during storage. HCN may volatilize. Prompt analysis is recommended. Addition of alkali to pH-11 and cold storage is the best approach when storage or transportation time cannot be avoided.

D Results should be expressed as μg or $\text{mg CN}^-/\text{l}$ of sample even though the HCN does not ionize to an appreciable extent except at high pH levels.

VIII EPA ANALYTICAL METHOD

Refer to EPA Methods Manual, 1971⁽⁷⁾ for information on method, sample handling and preservation.

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- 3 Serfass, E. J., et al. Analytical Methods for the Determination of Cyanides in Plating Wastes and in Effluents from Treatment Processes. *Plating* 39:267. 1952.
- 4 Ludzack, F. J., et al. Determination of Cyanides in Water and Waste Samples. *Anal. Chem.* 26:1784. 1954.
- 5 Finger, J. H. Laboratory Investigations No. 2. TA & I Section, Div. of Water Supply & Pollution Control. DHEW. Recovery of Simple Cyanide by the Serfass Procedure as Compared with the Williams Cuprous Chloride Method. April 17, 1964.
- 6 Standard Methods, 12th Edition, APHA. 1965.
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This outline was prepared by F. J. Ludzack, Chemist, National Training Center, MDS, WPO, EPA, Cincinnati, OH 45268.

COLORIMETRIC DETERMINATION OF IRON AND MANGANESE IN WATER

I INTRODUCTION

A Sources(1)

1 Nonacid ground waters

- a Iron occurs as soluble $\text{Fe}(\text{HCO}_3)_2$. The colorless clear, iron-bearing ground water develops a turbid red-dish brown color upon standing in air due to the oxidation of $\text{Fe}^{++} \longrightarrow \text{Fe}^{+++}$.
- b Manganese occurs as soluble $\text{Mn}(\text{HCO}_3)_2$.

2 Acid surface waters

- a Iron occurs as soluble FeSO_4 usually together with MnSO_4 , H_2SO_4 and $\text{Al}_2(\text{SO}_4)_3$. Acid waters generally do not cloud upon standing in air unless first neutralized.
- b Manganese occurs as soluble MnSO_4 .

3 Colored waters (found in the South)

- a Iron occurs in an organic chelated form in waters where color is due to the extraction of organic materials from decaying vegetation. Iron in this form does not precipitate when the water is aerated.
- b Manganese occurs in a chelated form.

4 Red waters

Iron occurs as insoluble, suspended $\text{Fe}(\text{OH})_3$ where the metal of piping, tanks and water mains has corroded.

B Effects (5)

- 1 Water treatment sand filters are clogged, and deposits of $\text{Fe}(\text{OH})_3$ and MnO_2 are formed on distribution

pipes. These deposits can break loose, be carried down to smaller pipes or valves and cause clogging and new corrosion.

- 2 Industrial use of water is affected, (e.g., paper and pulp or textile industries).
- 3 Domestic use of water is affected by staining of plumbing fixtures, forming of deposits on laundry, and imparting of unpleasant colors and tastes to beverages.

II DETERMINATION OF IRON

A Collection of Sample (7)

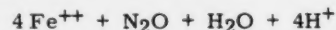
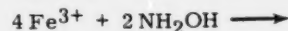
- 1 Iron contamination can come from the flaking of rust in pipes, or from a metal cap on the sample bottle.
- 2 Colloidal iron may adhere to the sides of a plastic sample bottle.

B Methods

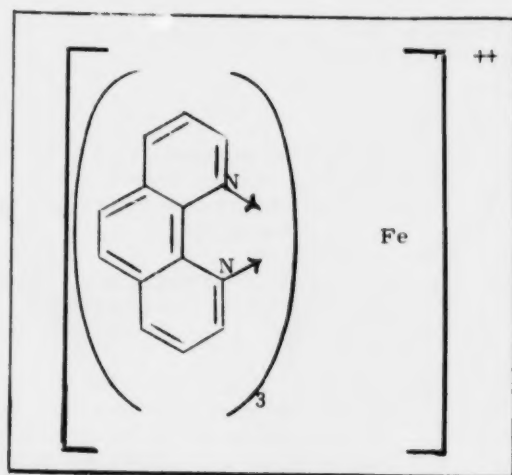
1 1,10-Phenanthroline (2,6,8)

a Reaction

- 1) Ferric iron is reduced to ferrous iron with hydroxylamine.



- 2) Three molecules of 1,10-phenanthroline chelate one ion Fe^{++} to form an extremely stable orange-red complex, which follows Beer's Law and exhibits maximum absorbance at 510 nm.



b Interferences (2)

There are several classes of ions which interfere with the 1,10-phenanthroline test for iron.

- 1) Ions which form iron complexes (e.g., phosphate polyphosphate, CN^-). This interference can be eliminated in the pretreatment of the sample by boiling with HCl.
- 2) Ions which form only slightly soluble complexes with 1,10-phenanthroline (e.g., Bi^{+++} , Ag^+ , Cd^{++} , Hg^{++} , Zn^{++}). This interference can be reduced by adding a large excess of 1,10-phenanthroline.
- 3) Ions whose own colors obscure the phenanthroline complex color (e.g., Ni^{++} , Co^{++} , Cr^{+++}). This interference can be reduced by using a blank containing an equivalent amount of the interfering ion present in the sample.
- 4) Oxidizing agents which prevent the complete conversion of $\text{Fe}^{+++} \rightarrow \text{Fe}^{++}$.

This interference can be reduced by adding a large excess of hydroxylamine.

c Extraction method (7)

Iron may be isolated from interfering ions by extracting it from aqueous, acid solution with diisopropyl ether. A subsequent aqueous extract of the iron from the ether is then used in the colorimetric determination with 1,10-phenanthroline.

d Procedure Information (7)

A detailed discussion of this procedure is in the current edition of Standard Methods.

Table 1. EFFECT OF ANIONS ON 1, 10-PHENANTHROLINE TEST
(All Samples Contained 2 mg/l iron)

Ion	Added As	Maximum Concentration mg/l	Maximum Interference % Fe	App. color pH Range
Acetate	$\text{NaC}_2\text{H}_3\text{O}_2$	500.0	None	2.0-9.0
Tetraborate (as B_2O_3)	$\text{Na}_2\text{B}_4\text{O}_7$	500.0	None	3.0-9.0
Bromide	NaBr	500.0	None	2.0-9.0
Carbonate	Na_2CO_3	500.0	None	3.0-9.0
Chlorate	KClO_3	500.0	None	2.5-9.0
Chloride	NaCl	1000.0	None	2.0-9.0
Citrate	$\text{C}_6\text{H}_8\text{O}_7$	500.0	None	2.0-9.0
Cyanide	KCN	10.0	2.0	2.0-9.0
Dichromate	$\text{K}_2\text{Cr}_2\text{O}_7$	100.0	Change hue	...
		20.0	None	2.5-9.0
Fluoride	NaF	500.0	1.6	4.0-9.0
Iodide	KI	500.0	None	2.0-9.0
Nitrate	KNO_3	500.0	None	2.0-9.0
Nitrite	KNO_2	500.0	None	2.5-9.0
Oxalate	$\text{Ni}(\text{C}_2\text{O}_4)_2$	500.0	None	6.0-9.0
Perchlorate	KClO_4	100.0	1.2	2.0-9.0
Phosphate (as P_2O_5)	$\text{Ni}(\text{H}_2\text{PO}_4)_2$	20.0	1.4	2.0-9.0
Pyrophosphate	$\text{Na}_4\text{P}_2\text{O}_7$	50.0	1.0	6.0-9.0
		20.0	1.0	3.0-9.0
Silicate	Na_2SiO_3	100.0	None	2.0-4.0
		50.0	None	2.0-9.0
Sulfate	$\text{Ni}(\text{SO}_4)_2$	500.0	None	2.0-9.0
Sulfite	Na_2SO_3	500.0	None	2.0-9.0
Tartrate	$\text{Ni}(\text{C}_4\text{H}_4\text{O}_6)_2$	500.0	None	3.0-9.0
Thiocyanate	KSCN	500.0	None	2.0-9.0
Thiosulfate	$\text{Na}_2\text{S}_2\text{O}_3$	500.0	None ^a	3.0-9.0

^a Transmittancy determined within 10 minutes after color developed. Iron reduced with hydroxylamine hydrochloride in all tests.

Colorimetric Determination of Iron and Manganese in Water

Table 2. EFFECT OF CATIONS ON 1,10-PHENANTHROLINE TEST
(All Samples Contained 2 mg/l Iron)

Ion	Added As	Maximum Concentration mg/l	Maximum Interference % Fe	Applicable pH Range
Aluminum	AlCl ₃	500.0	None	2.0-3.0
		250.0	1.4	2.0-5.0
Ammonium	NH ₄ Cl	500.0	None	2.0-9.0
Antimony	SbCl ₃	30.0	None	3.0-9.0
Arsenic	As ₂ O ₅	500.0	None	3.0-9.0
Arsenic	As ₂ O ₃	500.0	None	3.0-9.0
Barium	BaCl ₂	500.0	None	3.0-9.0
Beryllium	Be(NO ₃) ₂	500.0	1.3	3.0-5.5
Bismuth	Bi(NO ₃) ₃	None	...	...
Cadmium	Cd(NO ₃) ₂	50.0	1.0 ^a	3.0-9.0
Calcium	Ca(NO ₃) ₂	500.0	None	2.0-9.0
Chromium	Cr ₂ (SO ₄) ₃	20.0	None	2.0-9.0
Cobalt	Co(NO ₃) ₂	10.0	1.5	3.0-5.0
Copper	Cu(NO ₃) ₂	10.0	None	2.5-4.0
Lead	Pb(C ₂ H ₃ O ₂) ₂	500.0	None	2.0-9.0
Lithium	LiCl	500.0	None	2.0-9.0
Magnesium	Mg(NO ₃) ₂	500.0	None	2.0-9.0
Manganese	MnSO ₄	500.0	None	2.0-9.0
Mercury	HgCl ₂	1.0	None	2.0-9.0
Mercury	Hg ₂ (NO ₃) ₂	10.0	None	3.2-9.0
Molybdenum	(NH ₄) ₆ Mo ₇ O ₂₄	100.0	None	5.5-9.0
Nickel	Ni(NO ₃) ₂	2.0	None	2.5-9.0
Potassium	KCl	1000.0	None	2.0-9.0
Silver	AgNO ₃	None	...	...
Sodium	NaCl	1000.0	None	2.0-9.0
Strontium	Sr(NO ₃) ₂	500.0	None	2.0-9.0
Thorium	Th(NO ₃) ₄	250.0	1.5	2.0-9.0
Tin	H ₂ SnCl ₆	20.0	None	3.0-6.0
		50.0	None	2.5
Tin	H ₂ SnCl ₄	10.0	None	2.0-6.0
		20.0	None	2.0-3.0
Tungsten	Na ₂ WO ₄	10.0	Negligible	2.5-9.0
Uranium	UO ₂ (C ₂ H ₃ O ₂) ₂	100.0	None	2.0-6.0
Zinc	Zn(NO ₃) ₂	10.0	None	2.0-9.0
Zirconium	Zr(NO ₃) ₄	50.0	1.8	2.0-9.0
		100.0	2.2	3.0-9.0

^a 15.0 ml of o-phenanthroline in excess of the original amount added. Iron reduced with hydroxylamine hydrochloride in all tests.

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e Precision and Accuracy (7)

A synthetic unknown sample containing 300 µg/l Fe, 500 µg/l Al, 50 µg/l Cd, 110 µg/l Cr, 470 µg/l Cu, 70 µg/l Pb, 120 µg/l Mn, 150 µg/l Ag and 650 µg/l Zn in distilled water was determined by the phenanthroline method, with a relative standard deviation of 25.5% and a relative error of 13.3% in 44 laboratories.

2 2, 2', 2'' Tripyridine (4)

a Reaction

Two molecules of tripyridine chelate one ion of Fe⁺⁺ to form a reddish purple complex which obeys Beer's Law and exhibits maximum absorbance at 555 nm. Ethylenediamine is used to complex all of the heavy metals which might otherwise interfere.

b Procedure Information (7)

A detailed discussion of this procedure is in the current edition of Standard Methods.

c Precision and Accuracy (7)

A synthetic unknown sample with the same quantities of Fe, etc., used for the phenanthroline method evaluation was determined by the tripyridine method by six laboratories. The relative standard deviation was 62.4% and the relative error was 13.3%.

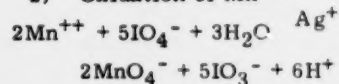
B Methods

1 Periodate (3, 6)

a Reaction

1) Elimination of interferences

2) Oxidation of Mn⁺⁺



The intensity of the permanganate color which is stable for several months is measured with a spectrophotometer at 525 nm.

b Interferences

There are several classes of ions which interfere in the periodate test.

1) Ions which precipitate as periodates or iodates (e.g., Ba⁺⁺, Pb⁺⁺, Hg⁺⁺).

This interference can be eliminated by maintaining an excess of strong acid (H₃PO₄) in which the periodates and iodates are soluble.

2) Ions whose colors obscure the MnO₄⁻ color (e.g., Fe⁺⁺⁺, Cu⁺⁺, Cr₂O₇⁼).

Prepare and run a corresponding blank or use the correction or turbidity method presented next.

3) Ions which cause turbidity in strong acid solutions (e.g., S₂O₃⁼, Tungstate, Bi⁺⁺⁺, Sn⁺⁺).

III DETERMINATION OF MANGANESE

A Collection of Sample

Acidification of a sample avoids the precipitation of MnO₂ and its consequent adherence to the sides of the sample container.

Table 3. EFFECT OF ANIONS ON THE PERIODATE TEST

(0.4933 mg of manganese in 250 ml solution)							
Ion	Concentration mg/250 ml	Apparent Change In Manganese Concentration %	Approximate Limiting Concentration mg/250 ml	Ion	Concentration mg/250 ml	Apparent Change In Manganese Concentration %	Approximate Limiting Concentration mg/250 ml
Acetate	60	Negligible	..	Nitrite	50	Decolorized	..
Arsenate	100 (As)	Negligible	..		50 (0.6 gram KIO ₄)	- 5.9	..
Arsenite	50 (As)	Change in hue	..		10	Decolorized	..
	20 (As)	-63.7	..		10 (0.6 gram KIO ₄)	- 4.4	..
	10 (As)	-36.1	0.0		3	-43.6	0.0
Borate	150 (B ₂ O ₃)	Negligible	..	Orthophosphate	200 (P ₂ O ₅)	Negligible	..
Bromide	50	- 7.0	10	Oxalate	50	Decolorized	..
	50 (0.6 gram KIO ₄)	Negligible	50		50 (0.6 gram KIO ₄)	Decolorized	..
Carbonate	200	Negligible	..		10	- 18.0	0.0
Chlorate	100	Negligible	..		10 (0.6 gram KIO ₄)	Negligible	10
Chloride	100	-35.0	..	Perchlorate	100	Negligible	..
	50	- 3.0	30	Pyrophosphate	300 (P ₂ O ₅)	Negligible	..
Citrate	50	Decolorized	..	Silicate	100 (SiO ₂)	Negligible	..
	50 (0.6 gram KIO ₄)	- 2.6	7	Sulfate	300	Negligible	..
Cyanide	100	-10.0	..	Sulfite	50	-71.6	0.0
	50	- 3.5	25		10	-12.5	..
Dichromate	10	Change in hue	..		10 (0.6 gram KIO ₄)	- 3.6	3
	2.5	Change in hue	0.0	Tartrate	50 (0.6 gram KIO ₄)	Decolorized	..
Fluoride	50	Negligible	..		10	- 3.1	25
Iodide	50 (0.6 gram KIO ₄)	Negligible	50		10 (0.6 gram KIO ₄)	- 2.0	10
	10	- 5.0	3	Thiocyanate	100	Change in hue	..
Molybdate	10 (Mo)	- 3.4	5		100 (0.6 gram KIO ₄)	- 6.2	10
Nitrate	200	Negligible	..		50	- 5.6	25
				Thiosulfate	1	Turbidity	0.0
				Tungstate	10	Precipitates	0.0
					10	Precipitates	0.0

Table 4. EFFECT OF CATIONS ON THE PERIODATE TEST

(0.4933 mg of manganese in 250 ml of solution)			
Ion	Concentration mg/250 ml	Apparent Change In Manganese Concentration %	Approximate Limiting Concentration mg/250 ml
Aluminum	100	Negligible	..
Ammonium	100	Negligible	..
Antimonous	100	Negligible	..
Barium	100	Negligible	..
Beryllium	50	Negligible	..
Bismuth	50 (20 ml H ₂ SO ₄)	Turbidity	0.0
Cadmium	100	Negligible	..
Calcium	100	Negligible	..
Chromic	50	Change in hue	..
	10	Change in hue	0.0
Cobaltous	50	+ 18.1	..
	10	+ 3.0	5
Cupric	50	Change in hue	..
	10	Change in hue	5
Ferric	100 (10 ml H ₃ PO ₄)	Negligible	..
Ferrous	5	Change in hue	0.0
Lead	100 (10 ml H ₃ PO ₄)	Negligible	..
Lithium	100	Negligible	..
Magnesium	100	Negligible	..
Mercuric	100 (20 ml H ₂ SO ₄)	Negligible	..
Mercurous	100	- 50.7	..
	50	- 1.1	50
Nickelous	50	Change in hue	..
	10	Negligible	10
Potassium	125	Negligible	..
Silver	100 (20 ml H ₂ SO ₄)	Negligible	..
Sodium	155	Negligible	..
Stannic	50	Negligible	..
Stannous	20 (20 ml H ₂ SO ₄)	Turbidity	0.0
Strontium	100	Negligible	..
Thorium	100	Negligible	..
Uranyl	50 (U)	- 2.0	50
Zinc	100	Negligible	..
Zirconium	100	Negligible	..

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Correction for turbidity or interfering color can be made by bleaching the permanganate color with a reducing agent and determining the absorption of the interferences.

- 4) Ions which are capable of reducing either IO_4^- or MnO_4^- (e.g., NO_2^- , $\text{C}_2\text{O}_4^{2-}$, Sb^{3+}).

This interference can be eliminated in the pretreatment by boiling the sample with H_2SO_4 , HNO_3 , and H_3PO_4 .

c Procedure Information⁽⁷⁾

A detailed discussion of this procedure is in the current edition of Standard Methods.

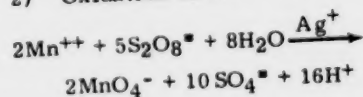
d Precision and Accuracy⁽⁷⁾

A synthetic unknown sample containing 120 $\mu\text{g/l}$ Mn, 500 $\mu\text{g/l}$ Al, 50 $\mu\text{g/l}$ Cd, 110 $\mu\text{g/l}$ Cr, 470 $\mu\text{g/l}$ Cu, 300 $\mu\text{g/l}$ Fe, 70 $\mu\text{g/l}$ Pb, 150 $\mu\text{g/l}$ Ag, and 650 $\mu\text{g/l}$ Zn in distilled water was determined by the periodate method, with a relative standard deviation of 36.0% and a relative error of 25.0% in 14 laboratories.

2 Persulfate⁽⁶⁾

a Reaction

- 1) Elimination of interference
- 2) Oxidation of Mn^{++}



If excess persulfate is present and organic matter is absent, the resulting color is stable at least 24 hours and is measured with a spectro photometer at 525 nm.

b Interferences

The interferences in the persulfate test and their removal correspond to those in the periodate test. However, when using the persulfate test, HgSO_4 can be used to complex Cl^- ion.

c Procedure Information⁽⁷⁾

A detailed discussion of this procedure is in the current edition of Standard Methods.

d Precision and Accuracy⁽⁷⁾

A synthetic unknown sample with the same quantities of Mn, etc., used for the periodate method evaluation was determined using the persulfate method by 33 laboratories. The relative standard deviation was 26.3% and the relative error was 0%.

IV SUMMARY

The U. S. Public Health Service has set the recommended limits for iron and manganese contents in finished potable waters at 0.3 mg iron/l and 0.05 mg manganese/l. Reliable quantitative tests for iron and manganese in trace amounts are important in the following areas:

- A Selecting New Sources for Water Supplies
- B Determining Water Treatment Processes
- C Preventing Corrosion in Pipelines

V EPA METHODS⁽⁹⁾

- A The Environmental Protection Agency, Office of Water Programs, Analytical Quality Control Laboratory, has compiled a manual of analytical methods which is to be used in Federal laboratories when analyzing surface waters.
- B In this manual, the use of atomic absorption is recommended for the analysis of both iron and manganese.

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This outline was prepared by B. A. Punghorst, Chemist, formerly with National Training Center, EPA and revised by A. E. Donahue, Chemist, National Training Center, MDS, WPO, EPA, Cincinnati, OH 45268.

DETERMINATION OF PHENOLICS

I DEFINITION AND SIGNIFICANCE

A Definition

The phenolic compounds in water chemistry are defined as hydroxy derivatives of benzene and its condensed nuclei. These occur in domestic and industrial wastewaters and in drinking water supplies.

B Phenol and chlorinated derivatives in water affect fish and water quality.

1 Fish

The threshold limit of toxicity at infinite time for certain species of fish is of the order of a few milligrams per liter. Some chlorinated phenols exhibit toxicity in concentrations as low as 0.2 mg/l.

2 Fish flesh tainting

Fish living in waters of lesser phenolic concentrations can acquire an unpleasant and obnoxious taste.

3 Water quality

The presence of as little as 14 $\mu\text{g/l}$ of the chlorinated phenols can impart a taste to drinking water.

II CHLORINE DERIVATIVES OF PHENOL CAUSING TASTE AND ODOR⁽¹⁾

A All chlorination products may contribute to the intensity of taste and odor.

B At maximum taste and odor intensity, the major contributor is a hitherto overlooked compound, 2,6-dichlorophenol.

C The chlorine-to-phenol ratio at maximum intensity of taste and odor is 2:1. The proportion of 2,6-DCP was greatest at the 2:1 chlorine-to-phenol ratio.

III PRESERVATION AND STORAGE OF SAMPLES

A Since phenolics are subject to oxidation, samples should be analyzed within 4 hours of collection.

B Samples can be preserved and stored up to 24 hours as follows:

- 1 Adjustment of pH to 4.0 with H_3PO_4
- 2 Aeration, if sulfides are present
- 3 Addition of 1.0 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$
- 4 Storage at $5-10^\circ\text{C}$

IV DETERMINATION OF PHENOLICS

A EPA Analytical Procedure⁽²⁾

The Environmental Protection Agency, Analytical Quality Control Laboratory has compiled a manual of analytical methods to be used in Federal laboratories when analyzing water and wastes.

The procedure recommended for phenolics involves distillation to separate out interferences, followed by the 4-aminoantipyrine colorimetric determination as presented in Standard Methods⁽³⁾ and in ASTM Standards⁽⁴⁾.

In most cases, phenol is used as a standard although Standard Methods⁽³⁾ includes a procedure for halogenated phenols which employs 2,4-dichlorophenol for the calibration curve.

B Other Analytical Procedures

Standard Methods⁽³⁾ also includes a tentative gas-liquid chromatographic method for samples containing certain phenolics present in concentrations greater than 1 mg/liter. Thin-layer chromatography has also been utilized for phenol and certain substituted phenols in raw surface water.⁽⁵⁾

V PRETREATMENT AND DISTILLATION OF SAMPLES^(3, 4)

Depending on interferences present, samples must be treated prior to the color determination.

A Pretreatment of Samples

- 1 Oxidizing agents, as detected by the odor of chlorine or by the starch-iodide test, are removed immediately after sampling by adding an excess of ferrous sulfate or sodium arsenite.
- 2 Oils and tars in a sample may contain phenolics. An alkaline extraction to remove these is required prior to adding CuSO_4 and distillation.

B Distillation

Most samples require a preliminary distillation to remove common interferences. The rate of volatilization of phenols is gradual so the volume of the distillate should equal that of the sample being distilled.

- 1 Phenols are distilled from non-volatile impurities.
- 2 Addition of copper sulfate to sample forms CuS , thus preventing the formation of H_2S or SO_2 which interfere with the determination. CuSO_4 also prevents biochemical degradation of phenolics.
- 3 Acidification of the sample with phosphoric acid assures the presence of the copper ion and prevents the formation of Cu(OH)_2 , an oxidizer of phenolics.

VI 4-AMINOANTIPYRINE DETERMINATION

A Applicability^(3, 4)

This method determines phenol, ortho- and meta-substituted phenols in which the substitution is a carboxyl, halogen, methoxyl, or sulfonic acid group. It does not determine those para-substituted phenols in which the substitution is an alkyl, aryl, nitro, benzoyl, nitroso or aldehyde group. Paracresol is an example of a common phenolic that is not sensitive to this determination.

B Method^(3, 4)

After pretreatment and distillation, the sample is reacted with 4-aminoantipyrine at pH 10.0 $\pm$ 0.2 in the presence of potassium ferricyanide (an oxidant) to produce colored antipyrine dyes. (See Figure 1).

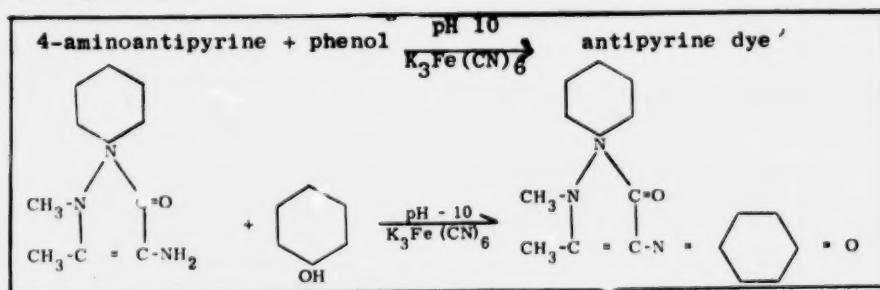


Figure 1

An absorbance measurement is made and the phenolic concentration is estimated using a calibration curve with phenol as a standard.

- 1 For original phenolic concentrations less than 1.0 mg/liter, the reaction product dyes are concentrated using the Chloroform Extraction Method. Absorbance is measured at 460 nm. and the results expressed as $\mu\text{g/liter}$ phenol.
- 2 For original phenolic concentrations greater than 1.0 mg/liter, the reaction product dyes are kept in the water solution for a Direct Photometric determination. Absorbance is measured at 510 nm., and the results are expressed as mg/liter phenol. This method is applicable to original phenolic concentrations up to 50 mg/liter.
- 3 Details of reagent preparation and the stepwise procedures can be found in the current editions of Standard Methods⁽³⁾ and ASTM Standards⁽⁴⁾.

C Variables

- 1 Sensitivity varies with pH. A buffer is used to maintain pH at 10.0 ± 0.2 to prevent the formation of antipyrine red and to minimize interference from aniline and undesirable enol-keto systems. (6, 7, 8)
- 2 The amounts of 4-aminoantipyrine and potassium ferricyanide used have a definite bearing on the amount of color developed. (7)
- 3 Temperature affects the rate of color change of the product dyes and of the blank. All materials used should be at the same temperature. (6, 8)
- 4 Direct sunlight or strong artificial light may have a bleaching effect on the colored materials. (9)

- 5 Filtration of the chloroform extracts removes water and increases their color stability to 3 hours. The mixtures measured in water solutions are not too stable and should be read within 30 minutes. (6)

VII PHENOL STANDARD

- 1 Because phenol is extremely sensitive to the 4-aminoantipyrine determination, the calibration curve used in the procedure is derived using phenol standards. These standards are prepared on the day of use by diluting a more concentrated stock solution of phenol.

The stock solution of phenol can be prepared by direct weighing. If extreme accuracy is required, this stock solution can be standardized using a bromate-bromide solution. (3)

- 2 Phenolics (substituted phenols) respond with various sensitivities to this test and produce colors of various densities. An example is this comparison of the absorbance values of the cresols to that of phenol: (7)

Compound	Absorbance Values Compared to Phenol(%)
phenol	100
orthocresol	74
metacresol	69
paracresol	3

- 3 Most phenolics sensitive to the test produce dyes with absorbancy maxima at or near the same wavelength so a photometric determination can be made.

VIII ACCURACY^(3, 7)

Results are an approximation and represent the minimum amount of phenol and phenolics present.

- 1 Only phenol is used as the color standard since it is impractical to prepare a standard containing a mixture of phenol and phenolics corresponding to each sample.
- 2 Different phenolics exhibit different sensitivities to the test.
- 3 Different phenolics produce differing shades of color which affect the final absorbance reading for the mixture.

IX PRECISION⁽⁴⁾

Reproducibility of results depends on on the interferences present in samples and on the skill of the analyst.

- 1 Using the Chloroform Extraction Method to concentrate color, six laboratories analyzed samples at concentrations of 9.6, 48.3 and 93.5 µg/liter. Standard deviation respectively, was 0.99, 3.1 and 4.2 µg/liter.
- 2 Using the Direct Photometric Method, six laboratories analyzed samples at concentrations of 4.7, 48.2 and 97.0 mg/liter. Standard deviation, respectively, was 0.18, 1.48, and 1.58 mg/liter.

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This outline was prepared by Audrey E. Donahue, Chemist, National Training Center, MDS, WPO, EPA, Cincinnati, OH 45268.

DETERMINATION OF SURFACTANTS

I NATURE OF SURFACTANTS AND SYNTHETIC DETERGENTS

A Definitions

1 Detergents

The dictionary states that the verb, deterge, means to wash off or to cleanse. Detergency denotes a cleansing power or quality.

Larson (5) characterizes a good detergent as a material which is water soluble, permits the water solution to penetrate capillaries by lowering interfacial tension (wetting action), breaks up or separates particles having agglomerated (dispersing action), and links the dirt or oil particles with the water (emulsifying action).

2 Surface active material (Surfactant)

This term is reserved for those organic compounds which exhibit detergent properties plus stability toward hardness. They are able to alter the surface or interfacial properties of their solutions to an unusual extent, even when present in low concentrations.

3 Synthetic detergents

The term, synthetic detergent, is not rigorously defined. In general, however, it is a material which contains a surfactant plus one or more builders.

4 Builders

A builder is a compound which is used to enhance the cleansing characteristics of a synthetic detergent.

Sodium sulfate, sodium silicate, sodium chloride, sodium tripolyphosphate, and carboxymethyl cellulose are examples of builders

which might be found in household or industrial synthetic detergents.

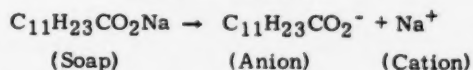
B Chemical Behavior of Surfactants

On the basis of their ionization in water, surfactants may be classified as anionic, cationic and nonionic.

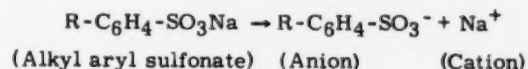
1 Anionic surfactants

These are the most widely used in the manufacture of synthetic detergents and are therefore the greatest contributors to pollution. They are characterized by the fact that they ionize in water to give an anion of large size and mass and a cation of small size and mass.

- a Soap exhibits detergent properties and ionizes as described above, but has become less popular for detergent use because of its lack of stability toward hardness.

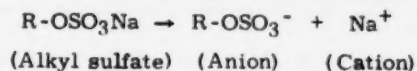


- b The alkyl aryl sulfonates represent a second type of anionic surfactant.

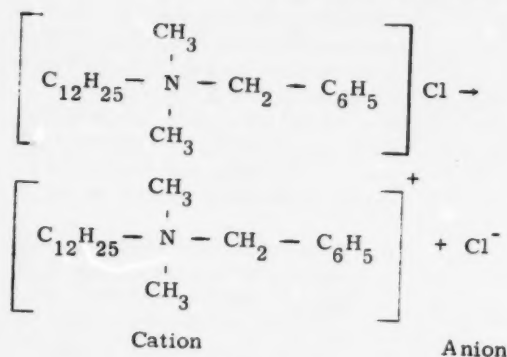


The C_6H_4 grouping represents a benzene ring; the R represents a chain of carbon atoms. If the chain is "straight" the alkyl aryl sulfonate is referred to as LAS. If the chain is "branched" the term ABS is applied.

- c Alkyl sulfates comprise the third type of anionic surfactant.

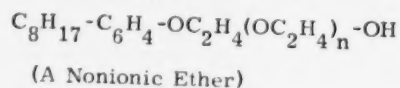


Compared to the anionic surfactants, the production of the cationics is small. They are noted for their germicidal properties and are used as sanitizing agents in connection with laundering and dishwashing. They ionize in water to give a cation of large size and mass and an anion of small size and mass.



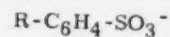
Interaction of cationic and anionic surfactants yields compounds which have neither germicidal nor detergent properties.

These do not ionize in water. They show little tendency to foam and are probably constituents of the so-called "controlled suds" detergents. Compounds, such as those illustrated, may have from 5 to 12 "ethoxy" or ether groups.



One of the functions of a good detergent

was stated to be its ability to link dirt or oil particles with the water (emulsifying action.) This linking ability can be considered by using an alkyl aryl sulfonate anion as an example.



The $R-C_6H_4$ portion of the anion is referred to as being hydrophobic (water repelling); i. e., it is insoluble in water, but soluble in grease and oil. The SO_3 portion of the anion is hydrophilic (water attracting); i. e., it is soluble in water, but is insoluble in grease and oil. In Figure 1 below, the rod represents the hydrophobic portion of the alkyl aryl sulfonate anion and the ball represents the hydrophilic part of the anion. In dilute water solution the anion orient themselves around the dirt and grease particles as shown in Figure 2. The dirt and grease particles thus coated (emulsified) show little tendency to coagulate and settle.

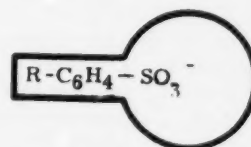


Figure 1.

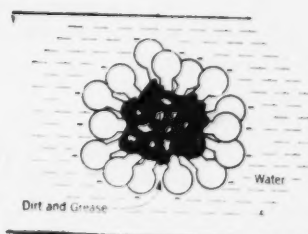


Figure 2. ACTION OF SURFACTANT ON DIRT PARTICLES⁽⁷⁾

II WASTES FROM THE USE OF SYNTHETIC DETERGENTS

A Sources of Wastes

1 Domestic wastes

The concentration of synthetic detergents (reported as MBAS) in raw sewage varies as shown in Table 1.

Table 1. MBAS CONTENT OF RAW SEWAGE (6)

City	MBAS in raw sewage (mg/l)
Oakland, California	4.4
Ponca City, Oklahoma	11.8
Cincinnati, Ohio	3.1
West New York, New Jersey	13.8

2 Laundry wastes

The average characteristics of a composite waste from a laundromat or small laundry operation, are presented in Table 2.

Table 2. LAUNDROMAT EFFLUENT (5)

COD (mg/l)	MBAS (mg/l)	pH (mg/l)	Suspended Solids (mg/l)
344 - 445	50 - 90	7.0 - 8.1	140 - 163

B Effects of Detergent Wastes on Water Quality

1 Foaming

MBAS levels in raw water at or below the USPHS Standards of 0.5 mg/l, do not cause foaming. At levels of 1 mg/l or above, foaming can occur.

Table 3 presents a summary of MBAS concentrations found in the Ohio River from 1954 to 1959, and reported by ORSANCO. (6)

Table 3. MBAS CONTENT OF OHIO RIVER WATER (1954 - 1959)

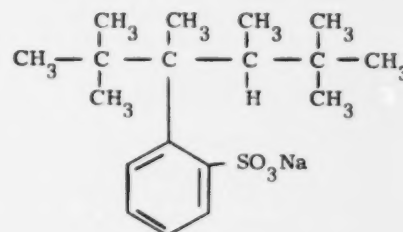
Value	MBAS (mg/l)
Median	0.12
Average	0.16
Weekly High	0.59
Weekly Low	0.01

2 Persistence in biological treatment

a Tetrapropylene ABS

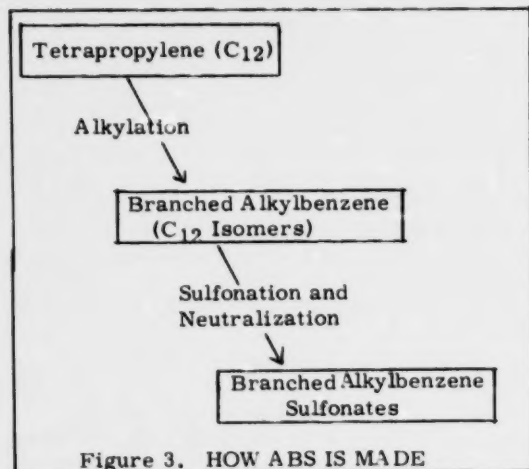
Studies have indicated that branched ABS compounds made by alkylating benzene with tetrapropylene (see Figure 3) are only 40-70% biodegradable in conventional activated sludge treatment. Consequently, they are known as biologically "hard" compounds.

It has been hypothesized (4) that the structure of the $C_{12}H_{25}$ benzene sulfonate isomer, which would be most difficult to degrade, would be:



b Linear alkyl sulfonate (LAS)

On the contrary, compounds made by alkylating benzene with n-paraffins (see Figure 4) have shown up to 98% biodegradability in conventional activated sludge treatment. These biologically "soft" surfactants known as LAS (linear alkyl sulfonate) compounds,



have been developed commercially to replace biologically hard ABS compounds.

c Nonionics

Ethoxylated alkyl phenol compounds containing less than 5 ethoxy groups have shown 90% to 95% biogradability. Larger numbers of ethoxy groups tend to increase resistance to biological treatment.

3 Eutrophication

The phosphate content of detergent wastes adds to the nutrient content of raw water. When the other essential nutrients are present, nuisance algal growth is promoted.

Compounds derived from amino carboxylic and hydroxycarboxylic acids are being considered as substitutes for the phosphate builders in order to decrease the nutrient load to streams and lakes.

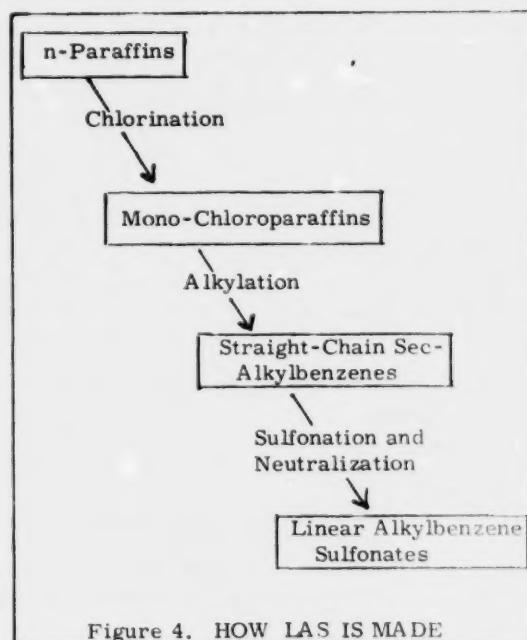
III ANALYTICAL METHODS

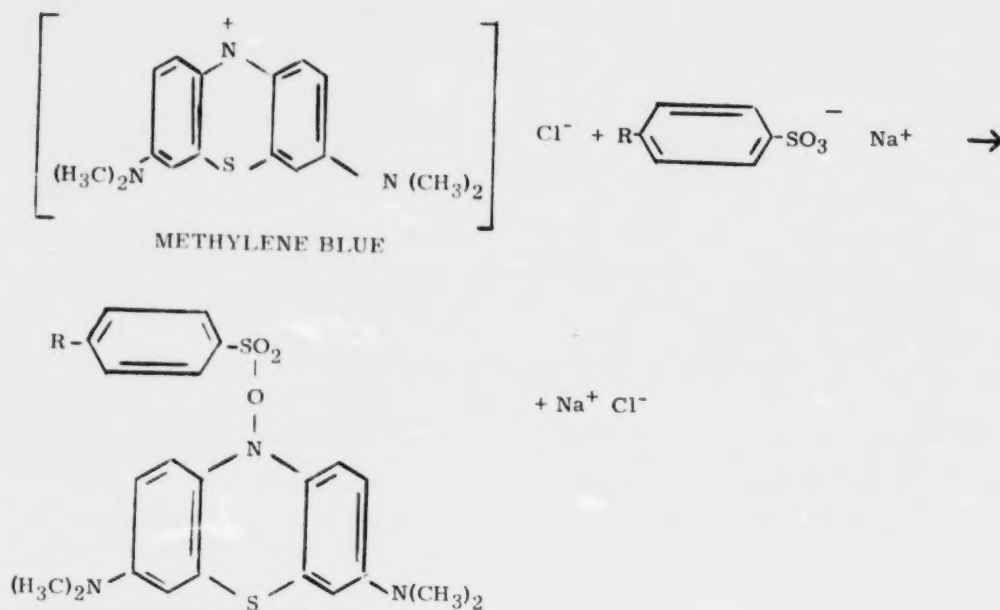
A Sample Collection

If a sample cannot be analyzed promptly, several procedures may be followed in order to preserve the sample.

- 1 Freezing will retard biological activity.
- 2 The addition of 0.8 mg concentrated H_2SO_4 /l of sample will also retard biological activity and thus preserve the sample.

It should be noted that losses of MBAS¹ have been found to occur when samples are stored in polyethylene containers. (2) It is believed that these losses are due to adsorption of MBAS¹ on the sides of the containers.



Figure 5. METHYLENE BLUE METHOD FOR ANIONIC SURFACTANTS⁽⁷⁾

B Anionics - Methylene Blue Method

1 Principle

Methylene blue reacts with anionic surfactants (and other chemical species) to form a blue-colored, slightly ionized salt which is soluble in chloroform. The color intensity of this product in this solvent is measured at a wavelength of 652 nm in a spectrophotometer. Range of application is 0.025-100 mg/liter for LAS.

Surfactants and other chemical species which react with methylene blue are classed as methylene blue active substances (MBAS).

- 2 This is the method of analysis for MBAS listed in the current EPA Methods Manual (10). Reagent

preparation and procedural details can be found in Standard Methods (11) and ASTM Book of Standards (12).

3 Interferences

- a Glassware used in this method must be acid cleaned so that it is free of even a trace of surfactant material.
- b Organic sulfates, sulfonates, carboxylates, phosphates and phenols will complex methylene blue, causing high results.
- c Inorganic cyanates, chlorides, nitrates and thiocyanates form ion pairs with methylene blue, again causing high results.

- d Organic compounds, especially amines, can compete with the methylene blue for the surfactant, causing low results. (e.g. proteins in sewage).

4 Precision and Accuracy

An Analytical Reference Service study in 1968⁽¹³⁾ obtained the following data:

- a On a sample of filtered river water, spiked with 2.94 mg LAS/liter, 110 analysts obtained a mean of 2.98 mg/liter with a standard deviation of 0.272.
- b On a sample of tap water spiked with 0.48 mg LAS/liter, 110 analysts obtained a mean of 0.49 mg/l with a standard deviation of 0.048.
- c On a sample of distilled water spiked with 0.27 mg LAS/liter, 110 analysts obtained a mean of 0.24 mg/l with a standard deviation of 0.036.

C Nonionics

- 1 Methods for nonionics have been based on formation of a Cobaltho-thiocyanate complex and subsequent colorimetric measurement. Burttschell⁽³⁾ proposed a more sensitive method (0.1mg/l) in which a complex with the heteropoly acid of tungsten is formed, hydrolyzed, and colorimetrically measured as WO₄ dithiol.

2 Infrared method (3)

The Soap and Detergent Association has developed a referee method which measures "true" ABS or LAS, as opposed to "apparent" ABS or LAS, with the methylene blue method. This method consists of extensive clean-up procedures, followed by IR identification.

ACKNOWLEDGMENT

This outline contains certain portions of a previous outline by Betty Ann Punghorst, former Chemist, National Training Center.

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This outline was prepared by C. R. Feldmann,
Chemist, National Training Center, and revised
by Audrey E. Donahue, Chemist, National
Training Center, DTTB, MDS, WPO, EPA,
Cincinnati, OH 45268.

VI. INSTRUMENTS AND INORGANIC ANALYSES

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PRINCIPLES OF EMISSION SPECTROSCOPY

I INTRODUCTION

The term "spectroscopy", in its broadest sense, refers to the study of the radiations of the electromagnetic spectrum. As no single instrument exists which will separate radiation from all parts of the spectrum, it is divided into regions which are related to the different types of instruments capable of producing or measuring waves of various lengths. A diagram of the spectral distribution of energy is shown in Figure 1.

Emission spectroscopy is concerned with that region wherein radiation can be sorted out into a spectrum by means of prisms or gratings, and includes the near infrared, the visible and the ultraviolet.

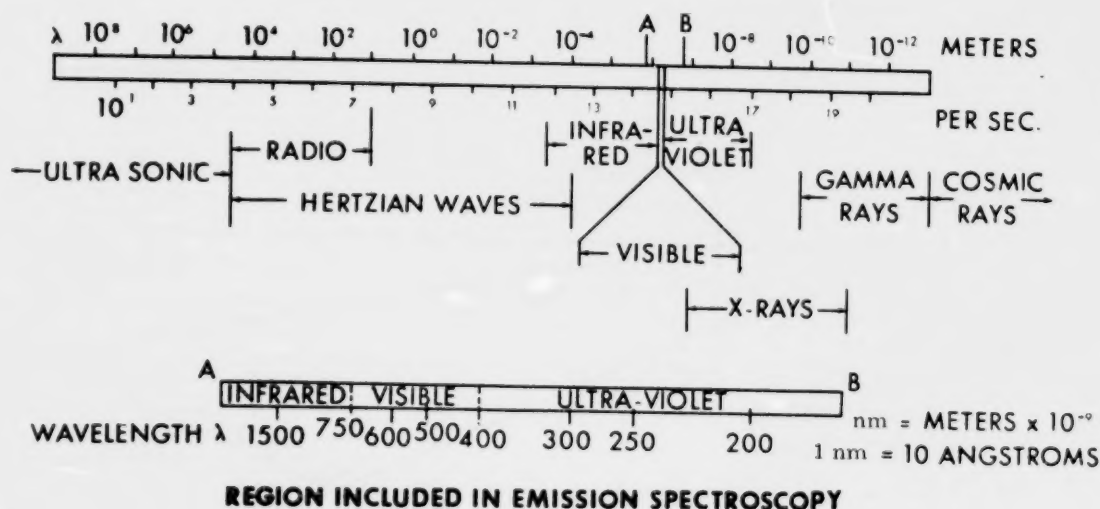
Emission spectroscopy has become an indispensable part of the modern chemist's analytical methods. With it he can analyze a wide variety of substances, both qualitatively and quantitatively, for trace elements regardless of valence states.

II THEORY

A Origin of Spectra - Excited atoms and atomic ions emit light of definite wavelengths. The excitation of multiple elements in a sample results in the simultaneous production of the spectra of all of these elements.

During excitation by a thermal or electrical source, the outer orbital electrons of an element absorb energy and rise to higher energy levels. These electrons then return spontaneously to their normal or ground state by a single jump or by a series of jumps. The energy emitted with each jump produces a spectral line of characteristic wave length and frequency for the particular chemical element. The combination of lines produced by the excited atoms of the element thus provides the emission spectrum of that element.

FIGURE 1
SPECTRAL DISTRIBUTION OF ENERGY



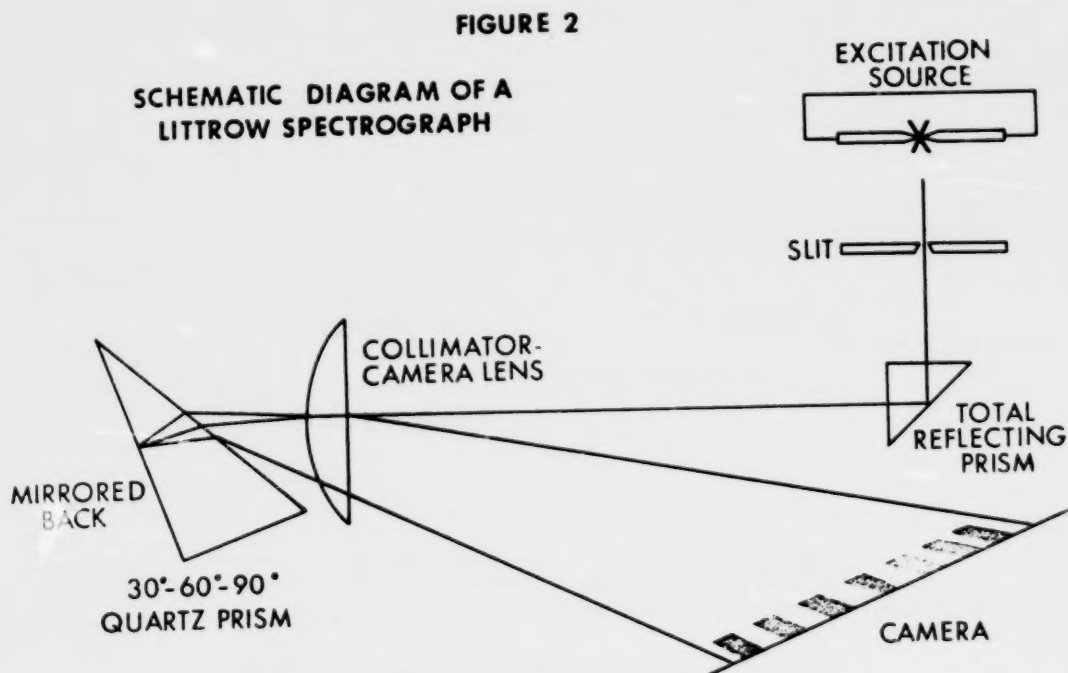
B Types of Spectra - There are three types of emission spectra.

- 1 The line spectra produced by highly excited atoms or atomic ions.
- 2 Band spectra which originate with highly excited molecules.
- 3 Continuous spectra which result when light is emitted by incandescent solids.

III INSTRUMENTATION

A The emission spectrograph consists of essentially four distinct functional parts: the excitation source, the slit, the optical system, and the recording system. A diagram of a Littrow type spectrograph is shown in Figure 2.

- 1 The production of line spectra for the detection or the determination of the elemental constituents in a sample requires an excitation source, such as an arc, a spark, or a flame.
- 2 The slit of a spectrograph permits only a narrow beam of light of mixed wave lengths to enter the instrument.
- 3 The optical system consists of a series of lenses and either a prism or grating to separate light rays of different wave lengths into the spectrum.
- 4 The recording system can be
 - a An eye piece such as used in a spectroscope.
 - b A photographic plate such as used in a spectrograph.



- c A photoelectric cell like that used in direct reading spectrographs.

IV SELECTION OF EQUIPMENT AND COST CONSIDERATIONS

A A decision concerning the advisability of installing a laboratory for spectrochemical analysis, whether in an industrial plant, research laboratory or health department, might rest upon four main considerations.

- 1 Whether the analytical problems encountered have a satisfactory solution other than spectroscopy.
- 2 The time urgency of the work.
- 3 The expected volume and diversity.
- 4 The cost of the initial investment, together with operating expenses.

V PROBLEMS AND TECHNIQUES ASSOCIATED WITH EMISSION SPECTROSCOPY

A After the equipment has been set up properly and is ready for operating, there are many parameters to be determined before the first samples are analyzed.

1 Source - arc or spark

There are several types of excitation sources available, as shown in Table 1. Generally, the type of sample and the precision and accuracy required for the analysis will govern which excitation method will be employed.

2 Electrode

The spectrographic electrode plays an important part in the analysis. Some important features are density, shape and purity.

Table 1. EXCITATION SOURCES

TYPE	PRECISION	SENSITIVITY	RANGE	USE
D. C. ARC	Fair	Excellent	0.00001 - 1.0%	Basic sources for general qualitative analysis - soils, ores, oxides, slags, ashes, etc. Highest sensitivity of detection for trace elements.
HIGH VOLTAGE A. C. SPARK	Excellent	Fair	0.01 - 30.0%	Most stable. Use for alloying constituents in metals. Solution techniques.
LOW VOLTAGE A. C. ARC	Good	Good	0.001 - 1.0%	Quantitative determination of residual impurities for low alloying constituents in metals.
SPARK IGNITED UNI - ARC	Good	Good	0.001 - 1.0%	Combines precision of the spark and the sensitivity of the D. C. arc.
HIGH VOLTAGE A. C. ARC	Fair	Excellent	0.00001 - 1.0%	Steadier than D. C. arc - Conductors and nonrefractory materials.

- a Density - The current and temperature obtainable are dependent on the density of the graphite.
- b Shape - The nature of the sample being analyzed will usually determine the shape and type of electrode.
- c Purity - Trace analysis requires that the electrodes be of very high purity.

3 Exposure conditions

Current, voltage, exposure time, etc., play an important part.

4 Photographic plates

The photographic emulsion most generally used for quantitative analysis is the S.A.#1. This emulsion responds to the spectral range from 2400 - 4400 Å. It has high contrast, low background density, and low granularity.

The S.A.#2 emulsion is also desirable for trace analysis when lower contrast, higher speed, and wide latitude are needed. S.A.#2 also covers the range from 2400 - 4400 Å. Other photographic emulsions covering the wavelength range from 2400 - 12,000 Å are available, each having specific features. The processing of these photographic plates is extremely important and the directions of the manufacturer should be followed closely.

B Qualitative Analysis

In spectroscopy, qualitative analysis is a relatively simple process, although it can be more time consuming than a quantitative determination, especially if a complete qualitative analysis is required. The presence or absence of more than sixty of the chemical elements can be determined readily by a simple inspection of the resulting pattern of spectral lines.

All elements give specific lines when sufficiently excited, which is the basis of qualitative spectrochemical analysis.

C Quantitative Analysis

Quantitative spectrochemical analysis is based on the fact that the amount of light emitted by an element present at very low concentration is directly proportional to the number of its excited atoms present, if all other factors are kept constant. The intensity of the spectral line of the analysis element (or degree of blackening of the photographed image of the line) in an unknown sample is compared to the intensity of the corresponding line in a standard sample to provide an estimate of the concentration of the element producing that line.

D Preparation of Standards

- 1 Since the major constituents of a sample are important factors affecting burning qualities of the arc and intensity of resulting spectral lines, it is important that the composition of the standards approximate the samples as closely as possible. This is accomplished by preparing a synthetic matrix to approximate the average composition of the samples minus the analysis element or elements.
- 2 Increasing concentrations of the analysis elements are added to the synthetic matrix and are sparked under the identical conditions used for the unknown samples for calibration purposes.
- 3 It should be emphasized that in quantitative spectrographic analysis, the best possible standards must always be prepared. A good spectrographer is only as good as his analyses, and his analyses are only as good as his standards. Spectrographic standard solutions are prepared from Reagent Grade chemicals. Oxides are prepared where possible; however, nitrates or chlorides will usually suffice.

Occasionally, it is necessary to use special spectroscopically pure grades of certain metals. Nitric and hydrochloric acids should be redistilled; double distilled water is also suggested where possible. Borosilicate glassware, cleaned with both chromic and nitric acids, is used throughout.

4 Internal standards

In quantitative analysis, the errors caused by temperature fluctuations and wandering of the arc are minimized by the use of the internal standard technique. This consists of comparing the intensity of a suitable line of analysis element in a standard and in an unknown sample to a certain selected line of another element whose concentration is fixed in all samples.

Since both the unknown and the internal standards are parts of the same sample, variations in time exposure, plate characteristics, and developing conditions will not affect the relative density of the two lines which are equal in intensity in the light source.

Working curves are established by plotting the intensity ratio of the analysis line to the internal standard line vs. the concentration of the analysis element.

E Accuracy

The average error in quantitative emission spectroscopy, using the photographic process, is generally between $\pm 5 - 10\%$. In the direct reading process, however, where photomultiplier tubes are substituted for the photographic plate, an average error of $\pm 2\%$ is possible. This accuracy is more than satisfactory, considering that determinations of trace elements are being made in the parts per billion range.

VI APPLICATION OF EMISSION SPECTROSCOPY TO WATER ANALYSES

A Trace elements in water originate from a variety of sources, but can be classified into three principal groups.

- 1 Elements contributed by soluble materials chemically weathered from rocks and soil.
- 2 Elements that are selectively concentrated by vegetation and find their way to surface waters following decay and run-off.
- 3 Industrial sources, especially those devoted to mining, alloying, and cleaning and plating of metals.

Such sources may contribute significant quantities of trace elements to surface waters in areas both populated and unpopulated. The detection and measurement of these trace elements are difficult with conventional analytical procedures because they are not adapted to large numbers of samples, and in some cases they are not sensitive enough. The use of spectrographic procedures for routine monitoring of raw waters, however, is admirably suited to the purpose since a large number of elements may be determined simultaneously with a high degree of accuracy.

B Trace elements, whether in finished or raw waters, are generally present in concentrations too low to measure directly with the spectrograph. Therefore, a means of concentration is necessary before the examination can be completed. This can be accomplished in several ways (e. g., evaporation, precipitation, ion exchange, etc.)

- 1 The method employed by the Environmental Protection Agency, Research and Monitoring Office, consists of evaporating a filtered volume of sample at low heat on a hot plate.

A volume of sample is chosen containing 100 mg of dissolved solids and evaporated to a volume of 5.0 ml or 20,000 mg of solids/l. (The dissolved solids consist for the most part of salts of sodium, potassium, calcium and magnesium; these are the materials for which corrections must be made later.) All samples, therefore, had the same final salt concentration, but the procedure produces varying levels of sensitivity for different sources because of the dissimilar initial volumes. Thus, the limit of detection for trace elements in the Columbia River is lower than for the Missouri River because the low dissolved solids in Columbia River waters permit a larger volume of sample to be evaporated.

- a This might be explained best by examining the detection limits shown in Table 2. For convenience, we have arbitrarily chosen 400 mg/l as an average TDS content of surface water, and the detection limits as shown are based on this figure. For waters of only 200 mg TDS/l, the limit would be halved; conversely, if the TDS is 800 mg/l, the limit is doubled.

Table 2. DETECTION LIMITS*, $\mu\text{g/l}$

Zinc	20	Copper	10
Cadmium	20	Silver	2
Arsenic	100	Nickel	20
Boron	10	Cobalt	20
Iron	10	Lead	40
Molybdenum	40	Chromium	10
Manganese	10	Vanadium	40
Aluminum	40	Barium	2
Strontium	4	Beryllium	0.1

*In water having 400 mg TDS/l.

- b The term "detection limit" is used to indicate the lowest concentration of the material that can be distinguished from background noise or interference. The limits, as shown in the table, are based on practical observations and are not mathematically contrived figures based on signal to noise ratios or other formulas.
- c When an element has not been positively identified in a sample, the detection limit, preceded by a "less than" sign (<), is used to report the analysis. It should be emphasized that the failure to detect a particular element does not mean that the element is absent; it may be present in the original sample but in a concentration below the stated detection limit. The complete spectrographic procedure has been published previously. Table 3 lists the results from analyses of over 1500 samples. The frequency of detection, the range, and the mean concentrations are given.

2 Accuracy

Recoveries were run with a composite of samples from the Missouri River. The composite was analyzed to determine what trace elements, if any, were present and at what concentrations. To a series of aliquots of this composite were added increasing concentrations of the analysis elements. The samples were acidified, evaporated on a hot plate, transferred to cylinders, and finally sparked under identical conditions. Scaler counts were converted to concentrations by using the data obtained from the synthetic matrix and the percent recoveries were calculated. A statistical treatment of this data showed a range of 80% to 113% at the 90% confidence level.

TABLE 3. SUMMARY OF TRACE ELEMENTS IN WATERS OF THE UNITED STATES*

ELEMENT	No. of Pos. Occurrences	Frequency Of Detection, %	Observed Values, $\mu\text{g/l}$		
			Min.	Max.	Mean
Zinc	1207	76.5	2	1183	64
Cadmium	40	2.5	1	120	9.5
Arsenic	87	5.5	5	336	64
Boron	1546	98.0	1	5000	101
Phosphorus	747	47.4	2	5040	120
Iron	1192	75.6	1	4600	52
Molybdenum	516	32.7	2	1500	68
Manganese	810	51.4	0.3	3230	58
Aluminum**	456	31.2	1	2760	74
Beryllium	85	5.4	0.01	1.22	0.19
Copper	1173	74.4	1	280	15
Silver	104	6.6	0.1	38	2.6
Nickel	256	16.2	1	130	19
Cobalt	44	2.8	1	48	17
Lead	305	19.3	2	140	23
Chromium	386	24.5	1	112	9.7
Vanadium	54	3.4	2	300	40
Barium	1568	99.4	2	340	43
Strontium	1571	99.6	3	5000	217

* 1577 Samples (October 1, 1962 - September 30, 1987)

** 1464 Aluminum Samples

TABLE 4. RECOVERIES OF TRACE ELEMENTS ADDED TO RIVER WATER

Element	Percent Recovery		Average
	Low	High	
Zn	86.	107.	94.1
Cd	100.	106.	103.5
As	80.	100.	93.5
B	82.	97.	88.9
P	70.	110.	91.8
Fe	85.	108.	94.1
Mo	75.	100.	83.7
Mn	94.	105.	99.1
Al	96.	125.	106.7
Be	101.	117.	109.2
Cu	96.	113.	100.8
Ag ^a			
Ni	90.	118.	103.0
Co	95.	103.	99.3
Pb	60.	93.	83.6
Cr	60.	105.	86.3
V ^a	100.	110.	104.0
Ba	70.	97.	81.7
Sr	81.	112.	95.1

^a Silver precipitated and was not included.

Table 4 lists the range of these percent recoveries along with the average recovery for each element.

C Analytical Reference Service Results

A sample containing 8 trace metals at known concentrations was analyzed by 66 participating agencies using a number of analytical methods. The mean results of all 66 laboratories, in addition to those laboratories employing spectrographic procedures, are shown in Table 5. It is apparent from this summary that the spectrograph can be used to splendid advantage on water samples.

SUMMARY

The theory and instrumentation of emission spectroscopy are presented. Consideration is given to the problems and techniques associated with trace element analysis in water using the Emission Spectrograph.

For the present time, atomic absorption is the method of choice for metals analyses in the EPA Methods Manual. (8)

When broad spectrum analyses are required, the Emission Spectrograph may be used with comparable accuracy and precision.

Table 5. SUMMARY OF RESULTS OBTAINED BY SPECTROGRAPHIC PROCEDURES ON ARS SAMPLE: WATER METALS, NO. 2.

Element	Amount added mg/l	Mean of 66 Labs	AMOUNT RECOVERED mg/l		
			Lab #71126	NWQN Lab	Lab #1615
Al	1.80	2.21	1.80	2.30	3.10
Cr	0.18	0.14	0.18	0.13	0.17
Cu	0.42	0.43	0.29	0.38	0.41
Fe	0.62 **	0.44	0.46	0.40	0.75
Mn	0.25	0.28	0.25	0.23	0.38
Cd	0.24	0.26	0.25 *	0.30	0.50
Zn	0.90	0.94	0.68	0.94	0.88
Pb	0.18	0.20	0.27	0.19	--

** Iron value was adjusted to mean value as suggested on Page 47 of ARS report.

* This result was erroneously included under Lab #7112A in the ARS report.

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This outline was prepared by J. F. Kopp, Chief, Metals Analyses Unit, Analytical Quality Control Laboratory, NERC, EPA, Cincinnati, OH 45268.

FLAME PHOTOMETRY

I PRELIMINARY

Flame photometry is the art and science of applying thermal energy (heat) to elements in order to effect orbital shifts which produce measurable characteristic radiations. The color of the emission and the intensity of brightness of emission permit both qualitative and quantitative identification.

The application of a very hot flame (2000°C or more) produces excitation of the element, caused by the raising of an electron to a higher energy level and is followed by the loss of a small amount of energy in the form of radiant energy as the electron falls back into its original position or to a lower energy level.

II INSTRUMENTATION

The six essential parts of a flame photometer are: pressure regulators and flow meters for the fuel gases, atomizer, burner, optical system, photosensitive detector and an instrument for indicating or recording output of the detector. These components are schematically shown in Figure 1.

A Atomizer and Burner

Numerous variations in atomizer and burner designs have been used. Figure 2 depicts the integral aspirator-burner used in Beckman instruments. The sample is introduced through the innermost concentric tube, a vertical

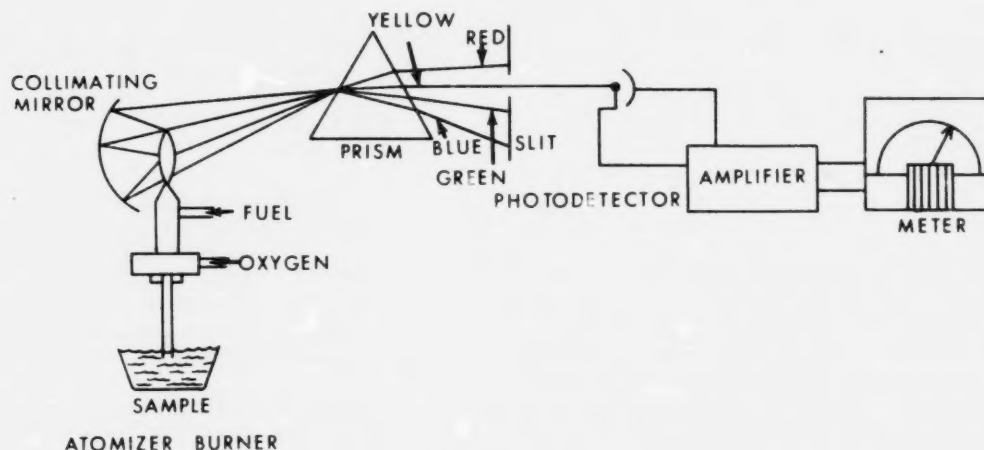


FIGURE 1. SIMPLIFIED DIAGRAM OF A FLAME PHOTOMETER

palladium capillary. A concentric channel provides oxygen, and its tip is constricted to form an orifice. Oxygen is passed from this orifice causing the sample solution to be drawn up to the tip of the inner capillary. There, the liquid is sheared off and dispersed into droplets. All droplets are introduced directly into flame, with a sample consumption of 1 - 2 ml per minute.

The main requirement of the burner is production of a steady flame when supplied with fuel and oxygen or air at constant pressures. In the Beckman aspirator-burner, a concentric channel provides oxygen to operate the atomizer and the flame. The additional concentric channel provides fuel for the flame.

B Optical System, Photosensitive Detector and Amplifier

The optical system must collect the light from the steadiest part of the flame, render it monochromatic with a prism, grating or filters, and then focus it on to the photosensitive surface of the detector. Use of filter photometers is least desirable due to their limited resolution. Flame spectrophotometers improve application as they will separate emissions in a mixture of metals, such as manganese lines at 403.3 nm and the potassium lines at 404.6 nm. Placement of a concave mirror behind the flame so that the flame is at the center of the curvature increases intensity of flame emission by a factor of 2.

Any photosensitive device may be used in a flame photometer. The detector must have a response in the portion of the spectrum to be used and have good sensitivity. The photomultiplier tube is the preferred detector for flame spectrophotometers.

The amplifier increases the signal from the phototube and improves resolution between close spectral lines. It also permits identification of elements present in samples when the concentration is very small.

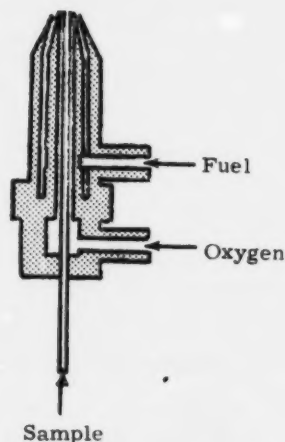


Figure 2. DETAILED DIAGRAM OF BURNER-ATOMIZER

III APPLICATIONS OF FLAME PHOTOMETRY TO WATER ANALYSES

Measurement of sodium and potassium in the past has been confined to complex, tedious and time-consuming gravimetric procedures. The flame technique enables the analyst to perform these determinations in a matter of seconds. If these metals alone were the only elements capable of measurement by flame photometry the use of the instrument could still be justified in a great many laboratories.

Other cations which may be detected and measured in waters and waste materials are calcium, magnesium, lithium, copper, and others. Table 1 includes those elements which may be measured with commercially available equipment, including ultra-violet and photomultiplier accessories.

Table 1 does not include wavebands which occur in the infrared spectrum. Sodium, for example, has an emission band at 819 nm which is not detectable with the common instruments.

Many other metals, including the rare earths, can be measured using the flame technique but they are not included in the table because

Table 1

	Wavelength	Approximate Sensitivity mg/l		Wavelength	Approximate Sensitivity mg/l
Aluminum	484.2	2	Lead	405?	2
	467.2	3		368?	2
	396.2	4		364?	3
Barium	553.6	0.3	Lithium	670.8	0.002
	493	0.4			
Beryllium	471	25	Magnesium	371?	0.1
	510	100		383?	0.1
Boron				285.2	0.2
	548	1	Manganese	403	0.01
	521	2		279*	1
Cadmium	495	3		561	2
	326.1?	2	Mercury	235.7*	10
	228.8*	40			
Calcium	422.7	0.003	Potassium	766.5	0.001
	622	0.004		404.6?	0.2
	554	0.01		344.7?	3
Chromium	425.4	0.1	Silver	338.3?	0.05
	360?	0.1		328.1?	0.1
	520	0.1	Sodium	589.3?	0.002
Copper	324?	0.01		330.3?	1
	327?	0.01	Strontium	460.7	0.02
Iron	372	0.2		681	0.01
	386	0.2		407.8	0.5
	373	0.3	Zinc	213.9*	500
				500	200

* = Ultraviolet spectrum

? = Doubtful detection in visible spectrum

the necessity for their measurement in water is a rare occurrence.

IV INTERFERENCES

A Spectroscopic Interferences

Energy at other wave lengths or from other elements than those intended to be measured may reach the detector. This problem is related to the resolution of the instrument and slit widths used.

Many of the instrumental difficulties are related to reproducibility of the flame. The quality and composition of the fuel affect the constancy and temperature of the flame which in turn influences the energy of emission. Likewise, slight variations in fuel pressures and ratios affect the reproducibility of the flame with reference to shape, temperature, background, rate of sample consumption, etc. In some cases, the temperature of the flame is the limiting factor in determining the presence of a metal. (The alkaline earth metals emit radiations at "low" temperatures, whereas other metals require very "hot" flames.)

Table 2 indicates temperatures obtainable with different fuel-oxidant mixtures.

Table 2.
Approximate Temperatures of
Fuel-Oxidant Mixtures for
Flame Photometer Use

Fuel-Oxidant	Approximate Temp. °C
Hydrogen - air	2100
Hydrogen - oxygen	2700 - 2800
Acetylene - oxygen	3100
Acetylene - air *	2000 - 2200
Propane - oxygen	2700 - 2800
Illuminating gas - oxygen	2800
Cyanogen - oxygen **	4900

* Undesirable because of carbon deposits.

** Used in research problems.

Emission reading of spectral lines always includes any contribution from the flame background emission on which the line is superimposed. When the photometer includes a monochromator, it is possible to read the background radiation in the presence of the test element. First, the line + background intensity is measured in the normal manner at the peak or crest of the band system. Next, the wave length dial is rotated slowly until emission readings decrease to a minimum at a wave length located off to one side or the other of the emission line or band. It is usually preferable to read the background at a lower wave length than the peak. Background reading is subtracted from the line + background reading.

Products of combustion may affect the characteristics of the flame or may affect the optical system by fogging or coating of lenses and mirrors.

B Factors Related to the Composition of the Sample

An element may be self-absorbing -- a phenomenon in which the energy of excitation is not proportional to the concentration of the element. As previously discussed, excitation is followed by loss of energy in the form of radiation as the electron falls back to its original position or to a lower energy level. During passage of radiant energy through the outer fringes of the flame, this energy is subject to absorption through collision with atoms of its own kind present in the ground energy level. Absorption of radiant energy weakens the strength of the spectrum line. Using the emission line at 589 nm for sodium, Figure 3 indicates that the line ceases to be linear at 13 mg/l. As the sodium concentration increases, the self-absorption effects become more pronounced. Sample dilution to permit reading on linear portion of the curve is often practiced.

Two or more elements present in the sample may produce radiant energy at

the same, or near the same wavelength. For instance, calcium at 423 nm and chromium at 425 nm could interfere with each other by additive effect. The correction may be to dilute out the unwanted metal or measure one of the emissions at a different wavelength.

The emission energy of one element may be enhanced or depressed by energies from other elements. This phenomenon (radiation interference) occurs when one element causes another to modify its actual emission intensity in either a negative or positive manner. Correction is obtained by dilution or by controlled interference addition.

Other types of difficulties encountered are too numerous to list here. In general, they may be overcome by improved instruments (high resolution, narrower slit openings, optics, flame adjustment) or possibly by special techniques.

Some inexpensive instruments, designed for limited use, may employ illuminating gas with air or propane with air as a matter of economy or convenience.

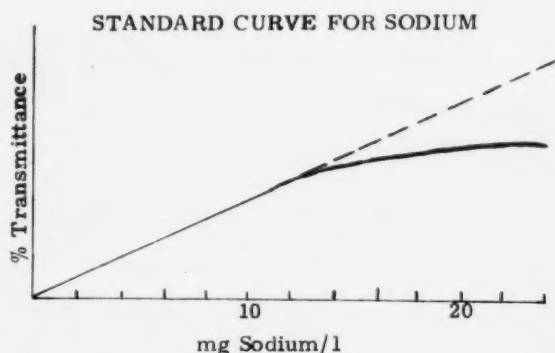


Figure 3

V TECHNIQUES

The following techniques are intended to serve as examples of current procedures in use for routine samples and for special samples where corrective procedures are indicated.

A Emission Intensity vs. Concentration

This is the classical procedure in flame photometry. Solutions (standards) containing known concentrations of test elements are compared with an unknown sample. This technique is applicable only when no interference is present.

B Radiation Buffers

For measurements of alkaline earth metals (sodium, potassium, calcium, magnesium) radiation buffers are prepared as solutions saturated with regard to each metal, respectively. A potassium buffer, for example, is prepared by saturating distilled water with sodium, calcium, and magnesium chloride. A calcium buffer in turn is saturated with sodium, potassium and magnesium chloride.

C Preparation of Radiation Buffers

For a sodium measurement, the buffer solution is added equally to samples and standards so that the interferences are alike for all readings, thereby cancelling each other (see Table 3).

D Instrument Improvement

Potassium emits energy bands at 766, 405, and 345 nm. The bands are at opposite ends of the spectrum and the 405 and 345 bands are not usable in the visible spectrum. The 766 line also loses sensitivity because of its proximity to the infrared region. Use of a red sensitive phototube or photomultiplier, however, permits measurement with an ordinary instrument at concentrations as low as 0.1 mg/l, or less. This approach is applicable to other elements also.

E Standard Addition

Equal volumes of the sample are added to a series of standard solutions containing different known quantities of test element, all diluted to the same volume (see Table 4). Emission intensities of the resulting solutions are then determined at the wavelength of maximum emission and at a suitable point on the flame background. After subtracting the background emission, the resulting net emissions are plotted linearly against the concentration of the increments of the standard solutions that were mixed with the unknown. The percent transmission of the mixture containing unknown sample and zero standard (distilled water) is doubled and the concentration corresponding to this point on the graph will be the concentration of the undiluted unknown sample. This can be explained algebraically in conjunction with Figure 4.

F Internal-Standard Method

The method consists of adding to each sample and standard a fixed quantity of internal standard element. The element must be one not already present in the sample. Lithium is usually the internal standard used. This method is most convenient when using instruments having dual detectors. The emission intensities of standards and samples are read simultaneously or successively depending upon instrumentation.

G Separation of Interferences

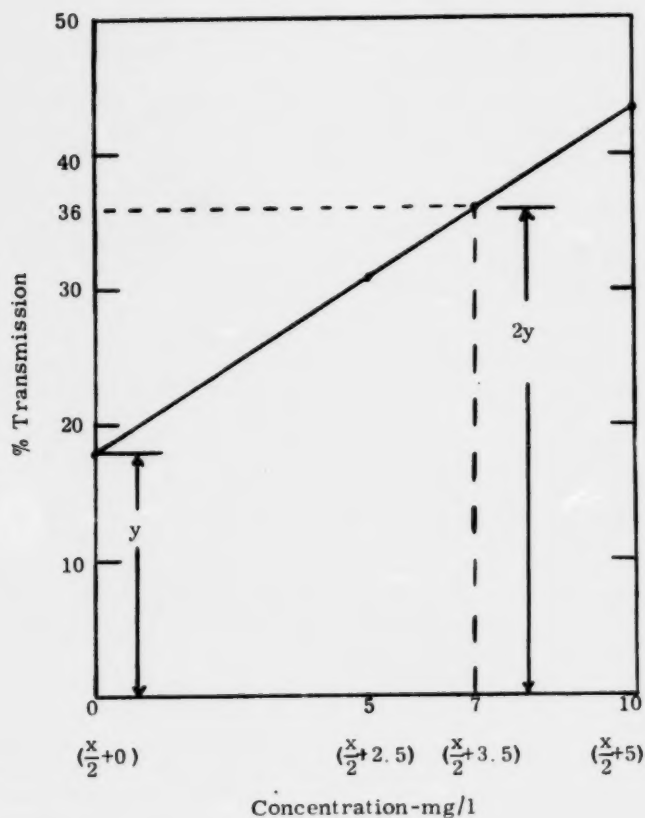
In cases where certain elements interfere, they may be physically removed, or the interference may be "blocked out" by reading the emission at different wavelengths. To measure lithium, for example, calcium, barium, and strontium are precipitated as carbonates of the metals. The lithium is retained in the filtrate and measured at a wavelength of 671 nm.

	NaCl	KCl	CaCl ₂	MgCl ₂
Sodium Buffer	-	+	+	+
Potassium Buffer	+	-	+	+
Calcium Buffer	+	+	-	+
Magnesium Buffer	+	+	+	-

Table 3

Conc. of standards	0.0 mg/l	5.0 mg/l	10.0 mg/l
Volume of standard added to sample	10.0 ml	10.0 ml	10.0 ml
Volume of sample used	10.0 ml	10.0 ml	10.0 ml
Concentration of element in each portion of mixture	$\frac{x}{2} + 0 \text{ mg/l}$	$\frac{x}{2} + 2.5 \text{ mg/l}$	$\frac{x}{2} + 5 \text{ mg/l}$

Table 4



Let x = concentration of element in unknown sample.

Then Y = % transmission of an equal mixture of unknown sample and zero standard, or

$$Y = \frac{x}{2} + \frac{0}{2} \text{ which simplifies to } 2Y = x$$

$$\therefore 2Y = \frac{x}{2} + 3.5 \text{ (from the example in Figure 4)}$$

$$\text{by substitution, } x = \frac{x}{2} + 3.5$$

$$\frac{x}{2} = 3.5$$

$$x = 7 \text{ mg/l}$$

Figure 4

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This outline was prepared by R. C. Kroner, Chief, Physical and Chemical Methods, Analytical Quality Control Laboratory, NERC, EPA, Cincinnati, OH 45268.

PRINCIPLES OF ABSORPTION SPECTROSCOPY

I INTRODUCTION

In any system employing principles of absorption spectroscopy, there are three basic components.

- A SOURCE of Radiant Energy
- B MEDIUM (Sample) which absorbs Radiant Energy
- C DETECTOR to measure the Radiant Energy transmitted by the Sample

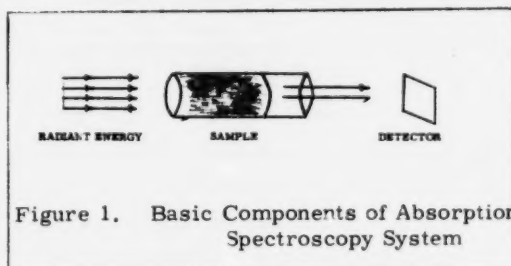


Figure 1. Basic Components of Absorption Spectroscopy System

II RADIANT ENERGY

A Wave Nature

- 1 The various forms of radiant energy have been arranged in a single schematic diagram referred to as the electromagnetic spectrum (see Figure 2). All of the energies which make up this spectrum may be represented graphically as waves. All waves move through space (and for most purposes air) at a constant velocity, 3×10^{10} cm/sec.
- 2 Three variable characteristics of individual waves serve to differentiate each from all other waves in the spectrum.

a The Wave Length

λ - The linear distance between the crests of two adjacent waves. (Units: distance/wave.)

b The Frequency

ν - The number of waves which pass a given point in a unit of time. (Units: waves/time unit.)

c The Wave Number

$\bar{\nu}$ - The number of waves which occur in a given linear distance. (Units: waves/distance unit.)

- 2 It is evident that more waves of short wavelength will "fit" into a given linear distance than would waves of a greater wavelength. Thus, waves having short wavelengths will have higher wave numbers. Mathematically, wave length is the reciprocal of wave number, if the same units of linear measurement are used in each expression. Since the velocity of all waves is equal and constant, it is also apparent that a greater number of waves of short wavelength can pass a given point in a unit of time than waves having a longer wavelength.

B Particle Nature

Planck conducted certain experiments which indicated that light has a particle as well as a wave nature. Energy rays can be said to consist of particles with a definite amount of energy. These particles or packets are referred to as photons or quanta. The energy (E) of each minute packet is given by Planck's equation,

$$E = h\nu \quad (5)$$

Where E = Radiant Energy in ergs

h = Planck's proportionality constant (6.6×10^{-27} erg sec.)

ν = Frequency in waves per second

Thus, it can be seen that the energy of a given photon is directly proportional to the frequency of the given Radiant Energy.

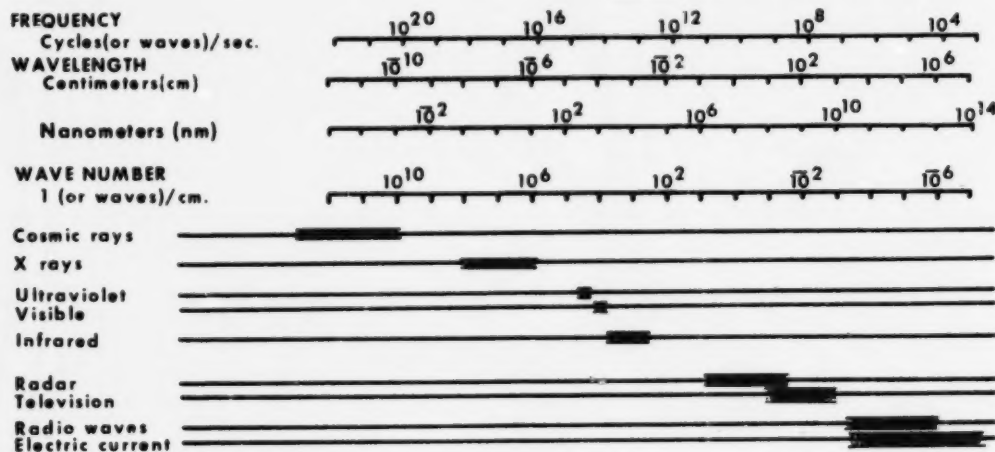
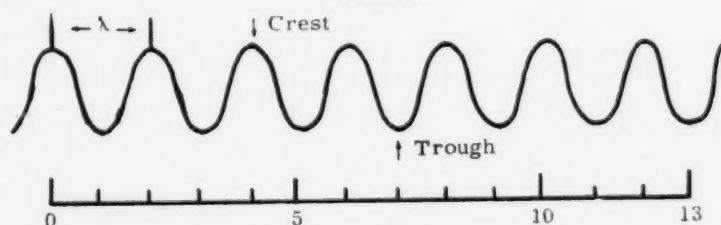


FIGURE 2

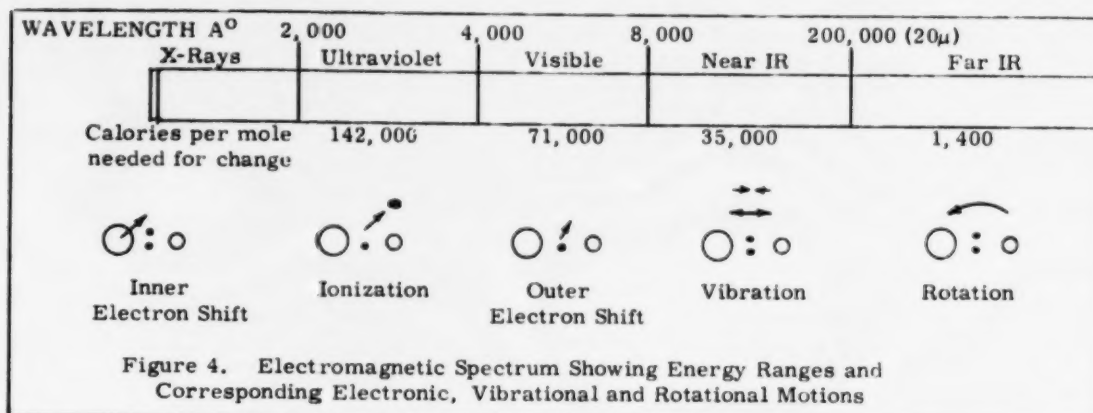


- | | | | | | |
|-----|---------------------------------------|---|------------------------------------|---|---|
| (1) | WAVE LENGTH | × | FREQUENCY | = | CONSTANT |
| (2) | λ | × | ν | = | c |
| (3) | $\frac{\text{distance}}{\text{wave}}$ | × | $\frac{\text{waves}}{\text{time}}$ | = | $\frac{\text{distance}}{\text{time}} = \text{VELOCITY}$ |
| (4) | λ (cm/wave) | × | ν (waves/sec) | = | $C = (3 \times 10^{10} \text{ cm/sec})$ |

Figure 3. Relationship of Wave Length and Frequency

III ABSORPTION OF ENERGY BY ATOMS AND MOLECULES

A Absorption of energies of given frequencies by atoms and molecules can be used as a basis for their qualitative identification. Absorption spectroscopy is based on the principle that certain displacements of electrons or atoms within a molecule are permissible according to the quantum theory. When radiant energy of the same energy required to bring about this permissible change is supplied to the molecule, the change occurs and energy is absorbed.



- 1 Displacement of electrons is a permissible change which can occur when energy of ultraviolet and visible frequencies strikes certain atoms and molecules.

- a Inner electron shift

Electrons located in the inner orbit of an atom may, when the proper frequency of radiant energy is available, shift to an orbit farther removed from the nucleus. This shift represents a change from a lower energy to a higher one. If this new position is unstable, the electron may revert to some position nearer the nucleus; the energy which was gained may then be emitted from the atom as part of its emission spectrum. The number of energy changes possible within an atom is a function of the number of electrons and the number of changes each may enter. Each possible change gives rise to a new spectral frequency. Since the frequency of radiation needed to accomplish such changes is of a high order of magnitude, the energy used is considerable in quantity.

Molecular aggregations often disintegrate in such circum-

stances; thus, these higher frequencies are used mainly for work with elements or very stable compounds.

- b Ionization

Under a specific frequency of radiation, an electron may be physically separated from its parent atom. This process has been termed ionization. A change of energy level of this magnitude requires less energy than the inner electron shift. Such changes are characteristic of those of the rare earths, inorganic ions, transition elements and many organic compounds under frequencies within the ultraviolet range.

- c Outer electron shift

The various orbital electrons in an atom may vary in the amount of energy required to shift them outwardly from the nucleus. For example, it requires less energy to shift an electron from a position more distant than it does to shift an electron outwardly from the inner orbit. Outer electron shifts occur readily in colored organic molecules for which

electronic transitions are made easier by the presence of chromophore groups which participate in resonance. Thus, the excitation of the delocalized outer electrons (pi electrons) is relatively easy and requires energy in the visible range.

- 2 Vibration of atoms within molecules is a permissible change which can occur when energy of near infrared frequency strikes certain organic molecules.

The atoms within a molecule are held together by attractive bonding forces. Atoms within a molecule are constantly moving toward and away from other atoms, but for purposes of theory can be said to have a certain "average" position.

The change in position of an atom in relation to another atom is called vibration. The mechanics of vibration require energy; the manner and rate of vibration of the atoms depend upon frequencies of electromagnetic radiation which strikes them. Therefore, a specific part of a molecule may absorb significant quantities of certain spectral frequencies. Such absorption will be reflected in the absorption spectrum of the compound. The energy requirements for this type of energy change are of a lower order of magnitude than those above; therefore, we would expect that the frequency required would be lower and the wave length longer. Such changes occur in organic compounds under infrared radiation.

- 3 Rotation of molecules is a permissible change which can occur when energy of far infrared frequency strikes certain organic molecules.

A molecule rotates around its symmetrical center. The manner and rate of rotation again depends upon the energy supplied to it.

Specific spectral frequencies of electromagnetic radiation can be employed to increase the rate of rotation. The used radiation is, in effect, absorbed and reflected in the absorption spectrum.

Organic molecules utilize infrared radiation while varying their rate and manner of rotation.

- B The Lambert-Beer Law provides the basis for quantitative analysis by absorption spectroscopy. It is a combination of the Bouguer (or Bouguer-Lambert) and Beer Laws.

- 1 Bouguer (or Bouguer-Lambert) Law

When a beam of monochromatic radiation passes through an absorbing medium, each infinitesimally small layer of the medium decreases the intensity of the beam by a constant fraction.

Mathematically:

$$\frac{-dI}{I} = k \, db \quad (6)$$

On integration and converting base e to base 10 logarithms,

$$\log \frac{I_0}{I} = A = Kb \quad (7)$$

-dI = increment by which incident monochromatic radiation is decreased (or absorbed) by the medium.

I = intensity of the radiation emerging from the absorbing medium.

k = proportionality constant whose value depends on the wave length and the nature of the medium; i.e., the solvent used if the absorbing medium is a solution, and the temperature.

db = increment thickness of the absorbing medium.

I_0 = radiation entering the medium.

$\log \frac{I_0}{I} = A = \text{absorbance}$
(optical density)

$K = 2.303 k$

b = length of radiation passing through the medium (i.e., the width of the cell, generally express in cm.)

2 Beer's Law

Each molecule of an absorbing medium absorbs the same fraction of radiation incident upon it regardless of concentration.

Mathematically:

$$\frac{-dI}{I} = k' dc \quad (8)$$

On integration and converting base e to base 10 logarithms,

$$\log \frac{I_0}{I} = K' c \quad (9)$$

k' = a proportionality constant whose value is governed by the same factors which determine the value of k .

dc = increment concentration of the absorbing medium.

$K' = 2.303 k'$

c = concentration of the absorbing medium (in the case of a solution c is generally expressed in moles/liter.)

3 Lambert-Beer Law

$$A = \log \frac{I_0}{I} = e b c \quad (10)$$

e = a constant obtained by combining K plus K' . When b is expressed in cm and c in moles/liter, e is called the molar absorptivity.

4 The term transmittance is sometimes used to express how much radiation has been absorbed by a medium.

$$\text{Transmittance (T)} = \frac{I}{I_0} \quad (11)$$

$$\% \text{ Transmittance (\%T)} = \frac{I}{I_0} 100 \quad (12)$$

The relationship between absorbance and transmittance is given by the expression:

$$A = \log \frac{I_0}{I}$$

5 The application of the Lambert-Beer Law to a problem involving quantitative analysis is made by the use of a calibration curve (or graph). See Figure 5.

Several standard solutions containing known concentrations of the material under analysis are "read" in the spectrophotometer. Figure 5 is prepared by graphing concentrations vs. corresponding absorbance readings.

If a straight line is obtained, the material is said to follow Beer's Law in the concentration range involved. The absorbance of the sample is then "read" and the corresponding concentration obtained from the calibration curve.

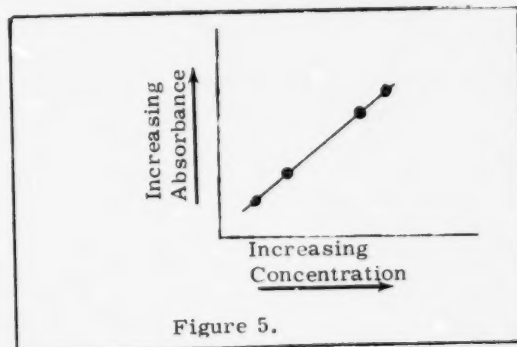


Figure 5.

ACKNOWLEDGMENT:

This outline contains certain portions from a previous outline by Betty Ann Punghorst, former Chemist, National Training Center.

RANGE	SOURCE OF RADIANT ENERGY	ABSORPTION BY SAMPLE		DETECTION OF RADIANT ENERGY TRANSMITTED
		CHEMICAL NATURE OF SAMPLE	TYPE OF SAMPLE CELL USED	
Ultraviolet	Hydrogen Arc	Inorganic ions and Organic Molecules	Quartz Fluorite	Photoelectric Cells Photographic Plates
Visible	Incandescent Tungsten Bulb	Colored Inorganic and Organic Molecules	Glass	Eye Photographic Plates Photoelectric Cells
Infrared	Nernst Glower Globar Lamp	Organic Molecules	Sodium Chloride or Potassium Bromide	Thermocouple

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This outline was prepared by C.R. Feldmann, Chemist, National Training Center, MDS, WPO, EPA, Cincinnati, OH 45268.

ATOMIC ABSORPTION SPECTROPHOTOMETRY

I INTRODUCTION

Atomic absorption spectroscopy has been well known to physicists and astronomers for more than 100 years. In 1850, Kirchoff took light from the sun and collimated it with a lens through the flame of an ordinary laboratory burner, and then passed the light through a prism which dispersed it into the characteristic visible spectrum with which we are all familiar. He then took a platinum spoon containing a sodium salt and introduced it into the flame. He observed that the yellow light that was present in the spectrum disappeared and in its place appeared the characteristic resonance lines of sodium. Since then astronomers have used the technique to detect and measure the concentration of metals in the vapors of stars. In 1953, Walsh⁽¹⁾ recognized its potential advantage over emission spectroscopy for trace metal analysis. He designed and built an analytically useful atomic absorption instrument. Shortly thereafter the advantages of atomic absorption instrumentation were recognized in the United States.

II THEORY

The basis of the method is the measurement of the light absorbed at the wavelength of a resonance line by the unexcited atoms of the element. Elements not themselves excited to emission by a flame may be determined in a flame by absorption provided that the atomic state is capable of existence.

At the temperature of a normal air-acetylene flame (2100°C) only about one per cent of all atoms is excited to emission in a flame; therefore absorption due to a transition from the ground electronic state to a higher energy level is virtually an absolute measure of the number of atoms in the flame, and the concentration of the element in the sample. Electrons will absorb energy at the same characteristic wavelength at which they emit energy. This is the principle upon which the technique of atomic absorption spectroscopy is based.

The advantages of atomic absorption spectroscopy as compared to emission spectroscopy are: (1) that atomic absorption is independent of the excitation potential of the transition involved and (2) that it is less subject to temperature variation and interference from extraneous radiation and interference from extraneous radiation or energy exchange between atoms.

Atomic absorption analytical apparatus (Figure 1) consists of a suitable source of light emitting the line spectrum of an element, a device for vaporizing the sample, a means of line isolation (monochromator or filter) and photoelectric detecting and measuring equipment.

If the detector is placed to receive only the resonance line of the element from the light source, measurement can be made of the absorption of resonance-line radiation on its passage through the vaporized sample. The magnitude of this absorption gives a measure of the concentration of free ground-state atoms of the element in the vapor and when referred to a calibration curve, provides a means of determining the concentration of the element in the sample.

III INSTRUMENTATION

The general arrangement of an absorption flame photometer is no different from an emission flame photometer except for the addition of a light source. An aerosol is introduced into a flame which is placed on the optical axis between the entrance slit of the monochromator and the monochromatic light source. Energy of the wavelength absorbed by the sample is provided by a source lamp whose emitting cathode is made of that element. This energy is passed through the flame and then through a dispersing device. A detector measures the absorbed and unabsorbed exciting radiation.

A Light Source

For the more volatile elements such as the alkali metals, mercury and thallium,

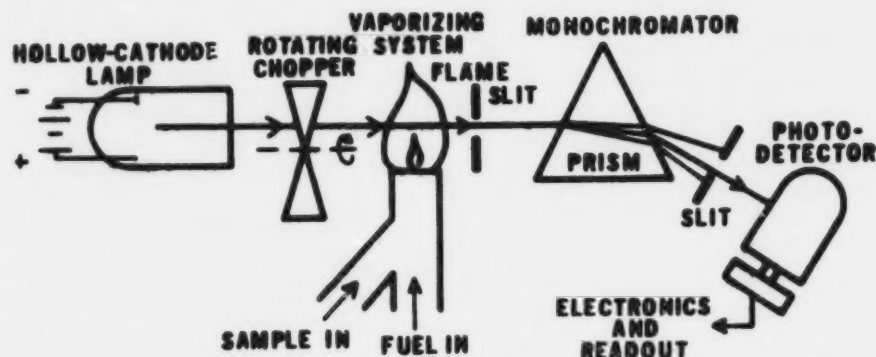


FIGURE 1

the most convenient source is the spectral vapor lamp, which consists of a closed glass or silica tube, into which are sealed oxide-coated electrodes, containing one of the rare gases and some of the appropriate metals. For line sources of the less-volatile elements, hollow-cathode discharge tubes have been found the most satisfactory. These consist of an anode and hollow cylindrical cathode (either composed of or lined with the appropriate metal) mounted in a sealed glass tube containing one of the rare gases (helium or argon).

Lamps are operated at low currents to improve linearity of response and maintain narrow emission lines.

B Vaporization of Sample

- 1 Atomic-absorption methods have been applied almost exclusively to the analysis of solutions and for this purpose flames, fed with a fine spray of the sample solution, similar to those employed in flame photometry are used.

The burner has two principal functions to perform:

- a It must introduce the sample into the flame.
- b It must reduce the metal to the atomic state.

- 2 Burners can be classified as:

a Total-consumption

Those which introduce the sample spray directly into the flame. (Figure 2)

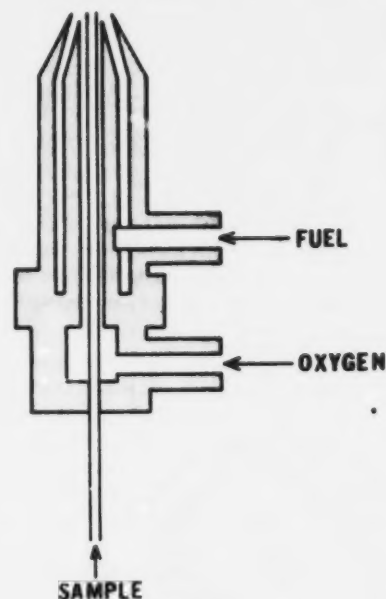


FIGURE 2

b Premix

Those which introduce the spray into a condensing chamber for removing large droplets. (Figure 3)

- 3 Flame shape is important. The flame should have a long path length (but a narrow width, such as a fishtail flame) so that the source traverses an increased number of atoms capable of contributing to the absorption signal.
- 4 The effective length of the flame may be increased by multiple passages through the flame with a reflecting mirror system, or by alignment of several burners in series.
- 5 The flame temperature need only be high enough to dissociate molecular compounds into the free metal atoms.

Typical flame temperatures are shown in Table I.

Table I

Fuel-Oxidant	Approximate Temp., °C
Nitrous Oxide - acetylene	3000
Hydrogen - air	2100
Hydrogen - oxygen	2700 - 2800
Acetylene - oxygen	3100
Acetylene - air	2000 - 2200
Propane - oxygen	2700 - 2800
Illuminating gas - oxygen	2800
Cyanogen - oxygen	4800

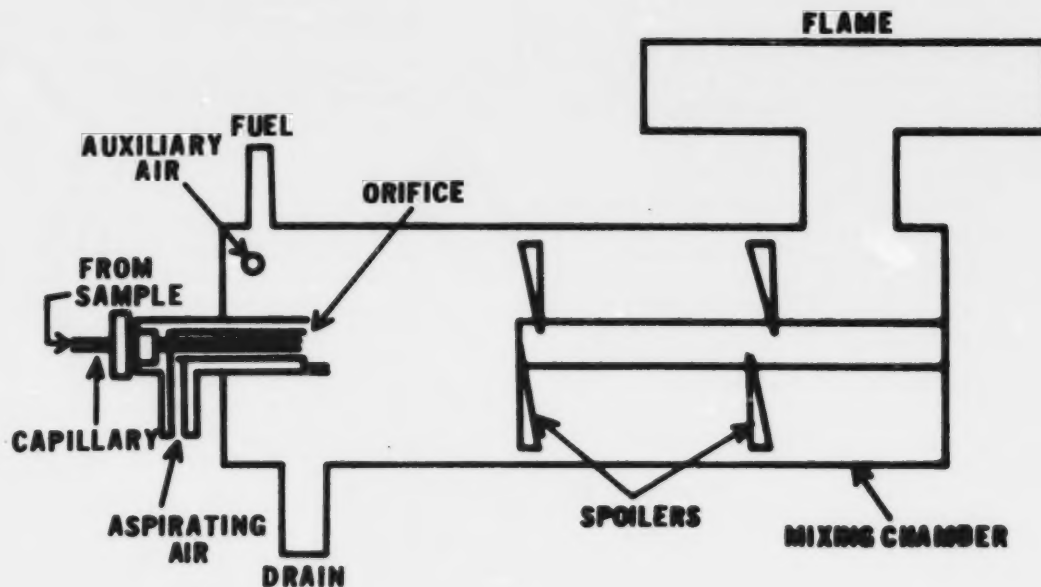


Figure 3

C Line Isolation

- 1 The use of a line spectrum of the element being determined, rather than a continuous spectrum, makes possible the use of monochromators of low resolving power or even filters. When a spectral lamp is used as a light source, it is only necessary to isolate the resonance line from neighboring lines of the light source or vaporized sample. The resolution of the method is implicit in the width of the emission and absorption lines.
- 2 To realize the full potentialities of the method, the strongest absorption line must be used. For elements with simple spectra, the resonance line arising from the lowest excited state is usually the line exhibiting strongest absorption.
- 3 Calibration curves depart from linearity at much lower concentrations in absorption work as compared with emission work. Curvature results partly from increased pressure broadening as the concentration of salt rises, but also depends on source characteristics, particularly self-absorption, and on the nature and homogeneity of the flame.

D Detection

- 1 Photo-electric detectors used in atomic absorption analysis need be no more sensitive than those used in emission analysis, since in the atomic absorption method, concentration of an element is determined by measuring the reduction in intensity of the resonance line emitted from a source of high intensity.
- 2 Single or double-beam circuits may be adopted for work with a single beam instrument, results are directly dependent upon source and detector stability. Both must be powered by separate power supplies. In a double-beam system small variations in the source signal are compensated automatically.

IV EVALUATION

A Sensitivity

- 1 For an air-acetylene flame of length 2 or 3 cm the lower limits of detection of elements having low resonance-line excitation potential (eg Na-K) are approximately equal in a single-beam atomic absorption and emission methods.
- 2 For elements having highly reversed resonance lines or resonance lines of high excitation potential, the atomic-absorption method has decided advantages over emission methods. Examples of elements in these categories are Zn, Mg, Fe and Mn.
- 3 A disadvantage of the atomic-absorption method, when compared with flame emission, is the lack of a quick and simple method of varying sensitivity to deal with solutions of widely varying element concentrations. The sensitivity of an atomic-absorption instrument is determined almost entirely by flame characteristics, notably length of light path through the flame.
- 4 A comparison of sensitivity obtained by emission and adsorption techniques is given in Table II.

B Precision

- 1 Precision of a single-beam atomic absorption instrument is primarily a function of the stability of light output from the spectral lamp. This in turn is dependent on the stability of the main supply and inherent stability of the lamp. The largest fluctuations are only ± 2 percent for the hollow cathode tube and sodium spectral vapor lamp. A double-beam instrument significantly reduces this error.
- 2 In common with flame-emission methods, atomic absorption is subject to "noise" from the flame and the detector. Changes in absorption caused by fluctuations in

Element	Sensitivity mg/l	
	Flame	A. A.
Aluminum	2	0.5
Antimony		0.2
Arsenic		1.0
Barium	0.3	1.0
Beryllium	25	0.05
Bismuth		0.2
Cadmium	2	0.01
Calcium	0.003	0.01
Cesium		0.05
Chromium	0.1	0.01
Cobalt		0.15
Copper	0.01	0.005
Gallium		1.0
Gold		0.1
Iridium		0.5
Iron	0.2	0.05
Lead	2	0.15
Lithium	0.002	0.005
Magnesium	0.1	0.003
Manganese	0.01	0.01
Mercury	10	0.5
Molybdenum		0.2
Nickel		0.05
Palladium		1.0
Platinum		0.5
Potassium	0.001	0.005
Rhodium		0.3
Rubidium		0.02
Selenium		1.0
Silver	0.05	0.02
Sodium	0.002	0.005
Strontium	0.01	0.02
Tellurium		0.5
Titanium		1.0
Thallium		0.2
Tin		2.0
Vanadium		0.5
Zinc	200	0.005

Table II

flame temperature are much less than those in emission because the strength of the absorption line is principally dependent on Doppler width whereas the intensity of emission from the flame is much more sensitive to temperature.

C Accuracy

This is shown by the types of interference found in flame emission and atomic absorption spectroscopy. There are three types:

1 Physical

Collision of atoms and electrons or atoms and molecules will transfer energy thus causing an enhancement or depression of analysis-line emission.

This has a large effect on flame emission analysis but has only a negligible effect on atomic absorption.

2 Radiative

Light from elements other than the one being measured pass the line isolating device (monochromator or filter). This occurs in flame emission work, for example, the interference of calcium and magnesium in sodium determinations. This interference is also encountered in atomic absorption using a D.C. system but is very small because of the large signal from the hollow-cathode tube. Radiative interference is eliminated in an A.C. system.

3 Chemical

Emission from an element in the flame is depressed by the formation of compounds, which are not dissociated at flame temperatures. This also affects absorption because the formation of temperature - stable compounds in the flame causes proportionate reduction in the population of ground-state and excited atoms.

Investigations to date suggest chemical interference is confined, almost entirely, to the alkaline-earth elements and that calcium absorption is more subject to this interference than is magnesium absorption.

Typical calibration curves are shown in Figure 5.

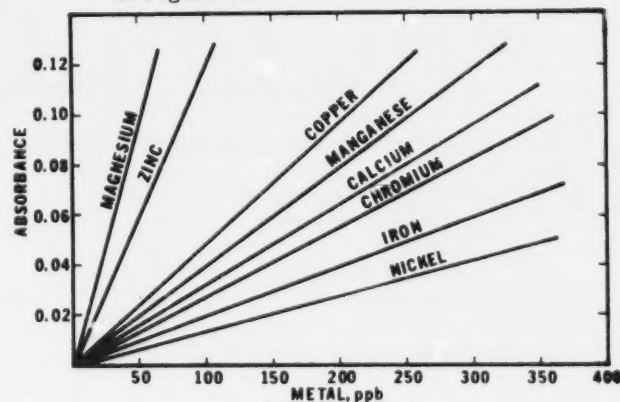


Figure 5

V REMOVAL OF INTERFERENCES AND CONCENTRATION OF SAMPLE

A Removal of Interferences

- 1 The methods for overcoming these interferences in atomic absorption are similar to those used in flame emission, namely, either separation of interfering ions or suppression of the interference by addition in excess of a substance that will prevent formation of compounds between interfering ions and the element being determined.

B Concentration of Sample

- 1 Organic separations can be used to concentrate a sample. Interferences are removed, as seen above, and also the organic solvent enhances the absorption.
- 2 Ion exchange has also been used successfully for concentrating samples for atomic absorption.

VI CONCLUSIONS

Atomic-absorption methods are as good as or better than emission methods, for elements to which they can both be applied, in sensitivity, precision and accuracy. They can be applied to a far wider range of elements than can emission analysis. The additional cost of hollow cathode discharge tubes is compensated by the greater range of analyses and greater reliability of results.

VII INSTRUMENTS AVAILABLE

A Perkin Elmer

- 1 Model 303 - double beam, AC -\$5,920.00
- 2 Model 290 - single beam, AC -\$2,900.00

B Beckman attachments for existing spectrophotometers.

- 1 Use with model D.U. and D.U. -2- single beam, DC - \$2,135.00

- 2 Use with model D.B. - single beam, AC - \$2,495.00

C Jarrell-Ash

- 1 Dual atomic absorption flame spectrometer - single beam, AC - \$5,800.00

D E. E. L.

- 1 Atomic absorption spectrophotometer - single beam, AC \$2,850.00.

ACKNOWLEDGEMENT:

Certain portions of this outline contain training material from a prior outline by Nathan C. Malof.

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This outline was prepared by Paul F. Hallbach, Chemist, National Training Center, MDS, OWP, EPA, Cincinnati, OH 45268.

VII. INSTRUMENTS AND ORGANIC ANALYSES

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TRACE ORGANIC CONTAMINANTS IN WATER

I INTRODUCTION

The subject of trace organic contaminants in water continues to receive increasing amounts of attention. Concurrently, the sources of these refractory materials are becoming more varied and complex. The problems associated with these substances are likewise increasing and satisfactory water treatment is becoming more and more difficult.

II SOURCES

A Man Made

- 1 Domestic wastes - In various stages of sewage treatment, discharged into rivers and streams.
- 2 Many complex industrial wastes, which have been accidentally or deliberately spilled or dumped.
- 3 Carrier solvents, such as those used in pesticide formulations.
- 4 Chemicals, such as fertilizers, which are applied directly to the land and water.
- 5 Petrochemicals.

B Natural

- 1 Extracellular products of algae by
 - a Diffusion of metabolic intermediates;
 - b By-products of metabolism;
 - c Hydrolysis of capsular materials.
- 2 Actinomycetes - microorganisms present in rivers/streams by their growth and decomposition cycles.
- 3 Bacterial decomposition of organic materials.

- 4 Run-off of land vegetation and soil.
- 5 Sediments, through decomposition and subsequent exchanges.

III CONCENTRATIONS

- A Waste outfalls contain from a few ppm up to 13% of organic contaminants.
- B Surface waters contain organic contaminants in concentrations ranging from less than ppb to several ppm.
- C Organic refractory substances cause serious problems in comparative trace amounts.

IV PROBLEMS

- A Taste and odor are usually the first effects noticed.
- B Increased chlorine and carbon demand interfere with coagulation and increase treatment costs.
- C Fouling of ion exchange resins and malodors in food and beverage industries.
- D Adverse effects on aquatic forms that support higher aquatic life, off-flavors in fish flesh, and direct toxic effects on fish.
- E Potentially long-term toxic effects and possible carcinogenic effects on humans.

V COLLECTION, ISOLATION AND IDENTIFICATION TECHNIQUES

A Sample Collection

- 1 Grab samples from 1 to 5 liters are adequate in many cases.

- 2 The carbon adsorption method (CAM) is the most common concentration technique.

- 3 The megasampler, a scaled-up version of the CAM, is used in special studies where large quantities of organic materials are needed.

B Isolation

- 1 The carbon samples are sequentially extracted with chloroform and ethyl alcohol to desorb the organic material from the carbon.

After extraction, the excess solvent is removed, and the samples are brought to dryness to yield:

- a) Carbon chloroform extract (CCE).
- b) Carbon alcohol extract (CAE).

- 2 The CCE is separated into broad classical groups by techniques based on solubility differences.

- 3 Further separations are made by such techniques as adsorption, paper, and thin layer chromatography.

C Identification

- 1 Tentative identification is normally made using gas chromatography, paper chromatography, thin layer chromatography, and fluorescent spectroscopy.
- 2 Positive identification generally requires the use of infrared spectroscopy, mass spectroscopy, mass spectroscopy in conjunction with gas chromatography, and nuclear magnetic resonance (NMR); sample sizes of a few mg are required for NMR.

VI SPECIFIC COMPOUNDS DETECTED AND IDENTIFIED USING THE CAM

- A These compounds have been detected and identified.

α -conidendrin; o-nitro-chlorobenzene; phenyl ether; ABS detergents; non-ionic detergents; 2-ethylhexyl phthalate; dieldrin; endrin; DDT; DDE; DDD; aldrin; heptachlor; heptachlor epoxide; BHC; parathion; methyl parathion; malathion; sevin; thiodan; chlordane; telodrin; technical and γ -chlordane; bis-(2-chloroethyl) ether; bis-(2-chloroisopropyl) ether; naphthalene; tetralin; styrene; acetophenone; ethyl benzene; 2-ethyl hexane; diisobutyl carbinol; phenyl methyl carbinol; 2-methyl-5-ethyl pyridine.

- B These compounds have been detected but not identified specifically.

Nitriles; amines; phenols; acids; ketones; aldehydes; alcohols.

VII TREATMENT AND REMOVAL PRACTICES

- A Plant treatment procedures, such as coagulation, sedimentation and filtration, are generally not too effective.

B Chemical Treatment

- 1 Copper sulfate used to control algae.
- 2 Oxidizing agents, such as chlorine, chlorine dioxide, ozone, and potassium permanganate, are used with varying degrees of success.
- 3 Activated carbon treatment removes organic substances by adsorption.

C Biological Treatment

- 1 Natural degradation in unsaturated soils and streams.
- 2 Biological oxidation of organic materials both in streams and acclimated systems.

VIII SUMMARY

The problems associated with trace organic contaminants in water are becoming more apparent as our needs and usage of water increase. The continued growth of the chemical industry, our increasing population, and the public's demand for more palatable water, emphasize even more the urgency of these problems. Developments have been made in the detection of these refractory materials and in their removal from water supply sources. It is apparent, however, that further advances in the collection, identification, and removal of these pollutants are needed to insure the public of high-quality water and water resources.

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This outline was prepared by R. L. Booth, General Analysis Group, Analytical Quality Control Laboratory, NERC, EPA, Cincinnati, OH 45268.

METHODS OF RECOVERING ORGANIC MATERIALS FROM SURFACE WATERS

Methods of recovering organic materials from water may be classified according to the degree of concentration required before the desired analytical procedure can be applied.

I CONCENTRATED SOLUTIONS

When the concentration is high and/or the methods are sufficiently sensitive (i.e., when only a minor degree of concentration is involved), the following methods should be considered.

- A Liquid-liquid extraction usually involves water and an immiscible organic solvent. Solvents should be investigated in the series of increasing polarity:

Aliphatic hydrocarbons
Aromatic hydrocarbons
Ethers
Chlorinated compounds
(CCl_4 , CHCl_3 , CH_2Cl_2)
Esters
Alcohols, amines, acids, etc.

Because of hydrolysis or decomposition, pH may be of critical importance; this point will be discussed at length in the discussion of Analytical Procedures. Inorganic salt concentration may also be important.

Remember the concept of the partition coefficient:

$$K = \frac{C_s}{C_w}$$

where K is the partition coefficient,

C_s is concentration in the extracting solvent

C_w is the concentration in water.

Continuous extractors must be used where the K value is not favorable to the extracting solvent. Continuous batch and counter-current extractors may be used.

Separatory funnels are available in a variety of sizes. Batch continuous

extractors are commercially available in sizes up to one liter or so, and can be readily assembled in larger sizes. Continuous counter-current extractors are convenient for up to 10-20 gallons.

- B Steam distillation can be used to strip a sample of small amounts of volatile organic compounds; in this case, the distillate usually must be further concentrated by liquid-liquid extraction. The organic compound should have at least a moderate vapor pressure at 100°C and should be almost insoluble in water.

A variation of this method is simple evaporation to concentrate non-volatile organic compounds.

- C Precipitants may be used: silver salts of acids, chloroplatinates or tetraphenyl boron, derivatives of amines, phenylhydrazine derivatives of ketones and aldehydes, etc.

- D Ion exchange may be used to concentrate acids and bases. The only limit to the volume of water to be filtered is the amount of inorganic salts in the water; these inorganic salts usually use up the exchange capacity too rapidly to make this method practicable.

II VERY DILUTE SOLUTIONS

To concentrate extremely dilute solutions, the carbon filter is the most useful method. The organic matter is adsorbed from aqueous solution and desorbed by an organic solvent. The great advantage is the large amount of water that can be put through a small filter; the disadvantages lie in the lack of quantitative adsorption and desorption.

A The Adsorption Process

- 1 The adsorption process involves an equilibrium in solution.

Adsorbed $\rightleftharpoons$ Unadsorbed

- 2 The Freundlich Isotherm is often useful in evaluating adsorption behavior.

$$\frac{x}{m} = kc^{\frac{1}{n}}$$

Units may be chosen for convenience, the larger the $\frac{1}{n}$, the stronger the adsorption. The constant k gives the $\frac{x}{m}$ value at unit concentration.

- x = weight of adsorbed compound
 m = weight of carbon
 k = constant = x/m when c is unity
 c = equilibrium concentration of organic compound in the liquid phase; it is measured directly.
 $\frac{1}{n}$ = constant determined by the slope of the isotherm

- 3 General Rule: Non-polar compounds are strongly adsorbed and polar compounds weakly adsorbed from water (e.g., mineral oil is more strongly adsorbed than glycine).

- 4 Adsorption will depend on the type of carbon, grain size, pH of solution, polarity of adsorbate, nature of the solvent, temperature, etc. If powdered carbon is not used, the column length and contact time must be chosen so as to permit efficient adsorption.

- 5 Desorption is simply the reverse reaction in the adsorption equilibrium and is influenced by the same factors as adsorption. It is usually necessary to use an organic solvent for desorption with an extended period of extraction.

B Setting up the Carbon Filter

- 1 In the next section, is a description of the procedure used in setting up and running carbon filters in our laboratory. However, it should be understood that elaborate apparatus is not required and that excellent results can be obtained with a piece of glass tubing closed at either end by a rubber stopper in which is inserted a piece of smaller glass tubing.

Such apparatus is adequate for testing small volumes (e.g., sewage or concentrated industrial wastes), although not convenient for handling thousands of gallons of water.

- 2 Wherever the investigation is concerned only with certain specific compounds, bench tests in small columns should be carried out in order to evaluate the performance to be expected from the filter.

III INSTALLATION OF EQUIPMENT AND COLLECTION OF CARBON FILTER SAMPLES

- A The carbon filter consists of a piece of pyrex glass pipe, 3" in diameter and 18" in length. The ends are fitted with brass plates and 3/4" galvanized nipples. A stainless steel screen is fixed in a neoprene gasket at both ends.

B Presettling, Prefilter, and Backwash

River water will frequently clog the carbon filter before the desired amount of water has been sampled. It is necessary to remove sufficient turbidity to permit the required amount of water to pass through the unit. This may require a presettling tank, and a prefilter containing sand and gravel. For waters having less than 100 ppm of turbidity, a presettling tank generally is not required. Tap water, of course, requires no prefiltering and may be passed directly into the carbon filter.

C Presettling Tank

A standard hot water tank connected with the inlet at the bottom and outlet at the top, with a clean-out tap at the bottom, can serve as a presettling tank. The outlet connects to the prefilter containing sand and gravel. The hot water tank should be flushed at frequent intervals to prevent large accumulations of solids. Open settling tanks can be used if care is taken to

prevent long detention times and biological action. With open tanks a pump will be required to move the water through the filter.

D Sand Prefilter

The sand prefilter consists of a standard piece of steel pipe, 3" in diameter and 3 feet long, threaded at both ends and equipped with 3" x 1" reducer couplings. Two disks of stainless steel screen are fitted to the inside diameter of the pipe. A simple way to prepare and hold the screen in place is to cut out a disk about the size of the outside diameter of the reducer and then push it into place so that it is below the threads. The screen is held tightly against the inside of the reducer. The pipe is packed with 6" of 1/8" gravel, 24 inches of 0.6 - 0.8 mm sand, and another 6" of 1/8" - 1/4" gravel. No free space is left in the pipe. The gravel should be packed by jarring while filling the pipe. This arrangement

provides a strainer rather than a filter with a movable bed. With such an arrangement, backflushing can be done without disturbing the filter. The construction details are shown in Figure 1. The dimensions may be varied according to local conditions.

IV FILTER ARRANGEMENT

A The presettling tank, the sand prefilter, and the carbon filter should be installed at the most convenient source of raw water. If less than 15 psi pressure is available, it may be necessary to pump the water through the system. A schematic drawing of a workable system is shown in Figure 2. Exact lengths of pipes, etc., are not given since these will vary with the local situation. Both the sand and carbon filters should be connected with unions at both ends for easy removal. A typical installation is shown in Figure 2.

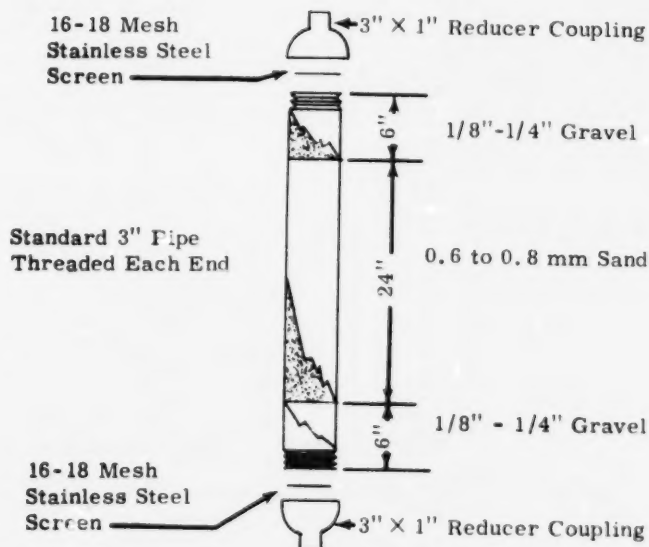


FIGURE 1 - Details of Sand Prefilter

B The raw water passes into the bottom of the sand prefilter, around into the bottom of the carbon filter. When the rate of flow through the system falls below 1/4 gpm, backwashing of the sand filter is necessary, using a high pressure source of water. A clean hose is connected to the top of the sand filter, the valve to the carbon filter is closed and the drain valve on the sand filter is opened. The sand is back-flushed until the water coming out is clear. The length of time between backwashings will vary. On the Missouri River, for example, it has been found that once every 24 hours is sufficient. After backwashing, connect the system as before and continue the sampling. Be sure to disconnect hose at top of sand filter after backwashing. A pressure gauge is inserted in the system to indicate when clogging is taking place in the carbon filter. Total pressure in the filter should not exceed 50 psi.

C A water meter located at the end of the system to prevent excessive fouling of the meter, is used to measure the volume of water samples. It is good practice to disassemble and clean the meter thoroughly after each run. A disk-type meter, 5/8"x3/4", is fairly satisfactory. A valve following the meter serves to throttle the flow, if necessary. A flow-regulating device may also be used for this purpose. The complete installation is shown in the schematic diagram in Figure 2.

D Fine carbon washes out of the filter when it is first started. A few gallons of water can be passed through the top connection and through the carbon filter drain before cutting in the meter to keep the meter free of carbon. The hose connection and drain on the carbon filter can be used to back-flush the carbon should it become clogged. This is a last resort and should only be used if absolutely necessary.

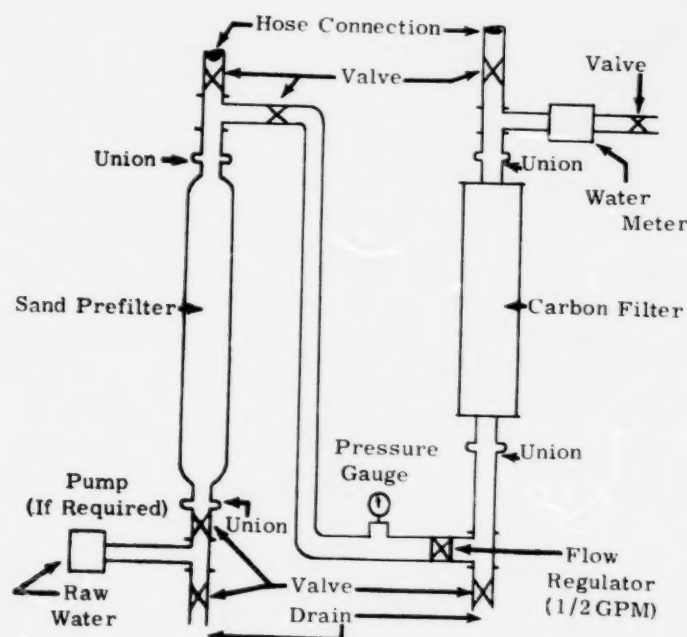


Figure 2 - Schematic Diagram of Piping Installation for Sand Prefilter and Carbon Filter

E The system outlined is not intended to remove all traces of turbidity from the water before passing through the carbon filter. Its purpose is to take out gross materials and most organisms, and to permit the required volume of water to pass through the carbon. The valve nearest the bottom of the sand filter is to be closed when it is desired to obtain a sample of raw water directly for other analyses. The drain valve is opened and, after flushing, the necessary raw water sample is collected.

F Pumping

- 1 If adequate pressure is not available for sampling raw water, it is necessary to pump through the filter system. It is important that the pump used should not contaminate the sample through oily packing or other sources. New pumps are oftentimes grease-coated and should be thoroughly cleaned before being put into service. If a lift of more than 12 feet is required, it will be necessary to use a jet-type pump. A model suitable for the conditions should be selected.
- 2 Before a new pump is put into service, it should be thoroughly flushed with hot water containing a little detergent. The pump should be operated against the minimum amount of head required to get the water through the filter. This may require by-passing of part of the flow.

G Collection of Sample

- 1 With fairly concentrated samples (sewage, industrial wastes, etc.), a small filter and a few gallons of water may be sufficient. Generally, for river sampling, water should be passed through a large filter at a rate of 1/4 - 1/2 gallon per minute, until up to 5000 gallons or more have been filtered. With highly turbid waters clogging may occur earlier. Although a suitable sample can be obtained sometimes with several

hundred gallons of river water, it is desirable to filter a minimum of 2000 gallons if at all possible. A flow-regulating device set at 1/4 or 1/2 gpm can be placed in the system ahead of the carbon filter. A suitable unit can be obtained from Dole Valve Company, 6201 Oakton Street, Morton Grove, Illinois. These devices generally need a minimum pressure of 15 psi for proper operation.

- 2 Since the purpose is to get a quantitative measure of organics in water, it is very important to have accurate flow measurements. If difficulties in flow occur, this should be noted on a log sheet. Meter readings should be recorded daily on log sheets and should be designated either in GALLONS or CUBIC FEET. If a satisfactory meter can not be obtained, the flow rate for a set volume (1 or 2 liters) can be determined at regular intervals.

H Precautions

The purpose of the carbon filter is to adsorb small amounts of impurities from the water. It is important to avoid contamination of the carbon from other sources. Hence, the following should be observed.

- 1 New galvanized fittings are usually coated with oil or grease. The oil should be removed with a wash in kerosene followed by a detergent wash before such fittings are used for making connection to the filter.
- 2 Ordinary organic pipe jointing compounds should not be used. Red lead (lead oxide) mixed to a paste with water, can be used for this purpose.
- 3 Plastic hose is to be avoided. If rubber hose is used in any connections it should be flushed thoroughly before being connected to the filter. Copper tubing is ideal for connections.

- I After the required volume of water has been run through the filter, the carbon is removed, dried, and extracted. The wet carbon is spread out in a thin layer on a metal sheet and air-dried for several days. The time needed for drying increases with the thickness of the layer. Warm air can be passed over the surface of the carbon to hasten the drying, but with the risk of driving off weakly held volatile substances.

V LABORATORY TREATMENT OF SAMPLES OBTAINED BY THE CARBON ADSORPTION TECHNIQUE: EXTRACTION PROCEDURES

The sample is collected by passing approximately 5000 gallons of water through a carbon adsorption unit. The unit is shipped to SEC, along with a daily record of the sampling activity and the carbon treated as described below.

A Preliminary Treatment

1 Records

- a Log sample in. List date received. Assign it a number.
- b Send daily record sheet to the laboratory where pertinent data is recorded on a data card (i.e., source, location, dates sampled, date received and total flow).

2 Drying

- a Remove wet carbon from the tube and dry it on copper, brass or stainless steel trays in an oven at 40°C. (n.b. Air circulated through drying cabinet should be prefiltered through carbon to prevent adsorption of foreign materials.) Drying requires about two days.
- b Store carbon samples in one gallon paint cans tightly closed.

B Solvent Extraction of Carbon

It is necessary that blanks be run on all solvents and on the carbon used for the collection of the sample.

1 Packing the extractors

- a Filter paper at bottom of soxhlet.
- b Glass wool (pre-extracted) 3" in depth to prevent carbon fines from passing into the pot. Add chloroform to wet the wool.
- c Carbon is added and packed. (n.b. Do not pack too tightly.)

2 Chloroform extraction

- a Add chloroform - about two cylinder volumes.
- b Extract for 35 hours.
- c Siphon and blow the bulk of the chloroform into the pot with air.
- d Remove the flask containing chloroform solution from the system. Concentrate to 250 ml by distillation and filter into a 300 ml Erlenmeyer flask. Evaporate to about 20 ml on a steam bath with a stream of air and transfer to a tared 5 dram vial. The remaining solvent is evaporated at room temperature without an air jet and the weight obtained.

3 Alcohol extraction

- a Remove residual chloroform from the carbon by one of the following methods.
 - 1) Blow warmed (40°C) prefiltered air upward through the carbon for about four hours.

- 2) Leach residual chloroform off the carbon with ethyl alcohol (95%) and distill off the chloroform alcohol mixture. Repeat until virtually pure alcohol remains.
 - 3) Remove the carbon from the soxhlet and air dry on trays. When dry, repack into soxhlet as before. (Chloroform vapors must be disposed of.)
- b Add sufficient ethanol (about two cylinder volumes) and extract for 24 hours.
 - c Concentrate the alcohol solution as in the case of chloroform, except that the final drying can be carried out on a steam bath with a stream of air.

The first method listed is the most satisfactory for our work here, mainly because a minimum of supervision is required and chloroform vapors are exhausted out the hoods.

C Extractable Materials

1 Calculation of concentrations

On most waters, it is convenient to compute the recovery in parts per billion (ppb) (i. e., $\mu\text{g/l}$), using the following formula.

$$\text{ppb} = \frac{\text{grams recovered} \times 10^6}{\text{gallons filtered} \times 3.785}$$

2 Infrared spectra

Infrared spectra are routinely run on both the chloroform and alcohol extracts.

D Special Applications

Other solvents may find use in performing extractions. If special applications are needed, a testing program is necessary to establish what solvents may best be used.

E Adsorption and Desorption

The effectiveness of adsorption and desorption varies for different materials and should be considered in interpretation of the results.

This outline was prepared by R. H. Burtshell, Chemist, formerly with AWTL, NERC, Cincinnati, OH and J. J. Lichtenberg, Pesticides Identification Group Leader, AQCL, NERC, EPA, Cincinnati, OH 45268.

PRELIMINARY SEPARATION OF EXTRACTS

I INTRODUCTION

As a general principle, it may be stated that the more sensitive modern analytical techniques (gas and other forms of chromatography, spectrophotometry, etc.,) require fairly pure samples to begin with. Also as a general principle, it may be said that the more widely different the purification steps employed, the greater the degree of purification.

Ion exchange, dialysis, crystallization, electrophoresis, etc., are useful but cannot be discussed here. We will cover briefly only three techniques.

A Distillation

B Partition

C Adsorption

II THEORETICAL BACKGROUND

A Distillation

- 1 Raoult's Law states that the partial pressure of A (P_A) in an ideal mixture of volatile solvents is

$$P_A = \text{mole fraction of A in liquid} \times \text{vapor pressure of pure A at the temperature of the system.}$$

The total vapor pressure (which, of course, sets the boiling point) is then $P_A + P_B + \dots$. This is the basis for studies of distillation. The question of non-ideal solutions is too complicated to discuss. Most physical chemistry texts go into it at length.

- 2 Partially miscible liquids are a special case, often discussed as "steam distillation" although co-distillation is a better term. Quoting from Glasstone, "As long as the two layers are present, irrespective of their relative amounts, the total

vapor pressure remains constant and the system boils at a definite temperature."

Besides the familiar case of steam distillation, non-polar organics can be co-distilled with polar liquids, e.g., insecticides with glycerol and polar compounds with non-polar solvents (i.e., mineral oil). The co-distillation can be done with superheated solvents, under vacuum. Where trace quantities of one component are involved, there may not be a two-phase system present and the solvent vapor serves only as a carrier gas. Here the solute can usually be recovered easily, however, so the method is worth consideration regardless of the mechanism.

In practical work, the three important points are the stability of the sample, its volatility, and the ease of recovery from the distillate.

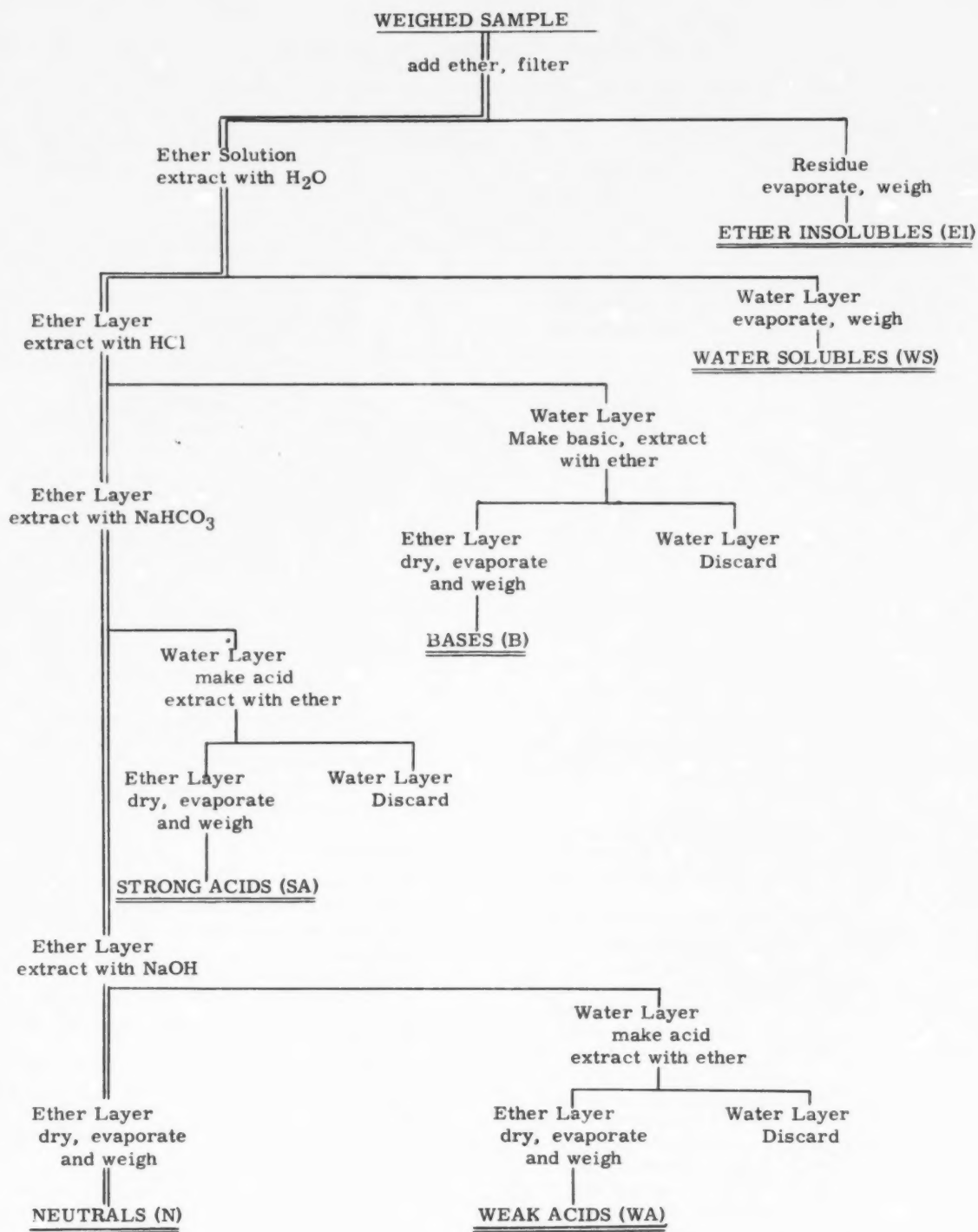
- 3 Distillation from acid and basic solutions may also be useful. The principle behind this will be discussed in the solubility separation method.

B Partition

Partition is the principle utilized in liquid-liquid extraction, paper chromatography and gas chromatography. The fundamental law is the Distribution Law: "A dissolved substance distributes itself between the layers of a two-layer system so that at constant temperature the ratio of the concentrations is also constant". The Law is properly applied only to dilute solutions, but a first approximation to the distribution ratio can be obtained from the ratio of solubilities.

$$K = \frac{C_s}{C_w} \quad K = \frac{S_s}{S_w}$$

Procedures for the Preliminary Separation of Extracts



K is a true constant but apparent discrepancies occur where the solute has different molecular weights in the two phases. Benzoic acid, for instance, is largely ϕCOOH plus some benzoate ion in water; in benzene it is the dimer $(\phi\text{COOH})_2$. K then applies only to the species common to both layers, ϕCOOH , and ignores the dimer.

Most texts on qualitative organic analysis contain a great deal of practical information on solubilities, while the usual undergraduate physical chemistry texts cover the essentials of the theory.

C Adsorption

There are two kinds of adsorption to be considered: physical (van der Waals) adsorption, and chemisorption. The theory of adsorption from solution is not as well understood as adsorption of gases but we can make a few comments.

Physical adsorption depends on the relatively weak van der Waals forces due to electrostatic attraction of dipoles, it being assumed that non-polar molecules act as oscillating dipoles; i.e., the nuclei of atoms and molecules form oscillating dipoles with the electrons. Since the bonds are weak, adsorption can be made reversible under proper conditions.

Chemisorption is thought of as actual chemical reaction, and the resulting bonds are much more difficult to break. Losses due to "irreversible adsorption" may therefore appear; this is particularly true of carbon, less so of silica and alumina.

III SOLUBILITY SEPARATION OF EXTRACTS

In the next section, we will describe a laboratory procedure which we have found very useful in handling carbon filter extracts. The extracts are split into acid, basic, and neutral fractions, and the neutral fraction is further separated by adsorption chromatography.

This procedure has been extremely useful where the fractions are weighed and infrared spectra made. However, where volatile compounds are to be looked for by gas chromatography, it is almost imperative that a steam distillation step be included. Otherwise the mass of heavy, probably polymeric, material present causes much interference.

Really good clean-up methods are likely to be highly specific and need to be "tailor-made" to suit the situation. Since this lecture is concerned only with the "preliminary" purification, we have omitted a discussion of precise analytical partition and adsorption columns (thin-layer, ion exchange, paper and gas chromatography, gradient elution methods, etc.) Such methods constitute a second and higher degree of purification, but ordinarily require that they be preceded by a "rougher" primary purification.

IV NOTE ON LOSSES OF VOLATILE COMPOUNDS

It may not be generally recognized, but it is quite true that heavy losses of even moderately volatile compounds occur if one tries to evaporate off all of the solvent when preparing to weigh an extract. We have had losses of over 50% in attempting to carry out what we thought were very careful evaporations of ether from 10 to 20 mg amounts of phenols.

For this reason, we suggest that no extract or fraction thereof be evaporated down for weighing unless it is known to be completely non-volatile. This precaution becomes more necessary as the purity of the sample increases, because non-volatile impurities act as "solvents" to reduce the amount of loss (according to Raoult's Law).

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This outline was prepared by R. H. Burtschell, Chemist, formerly with Waste Identification and Analysis Program, Advanced Waste Treatment Research Laboratory, NERC, EPA, Cincinnati, OH 45268.

LABORATORY PROCEDURES FOR THE PRELIMINARY SEPARATION OF EXTRACTS

I STEAM DISTILLATION OF EXTRACTS

Steam distillation need be only a very simple procedure. Put a gram or two of crude or partially purified sample in a small flask containing 50 - 100 ml of distilled water. Set up for ordinary distillation and boil off most of the water. Set up for ordinary distillation and boil off most of the water. If necessary, add more water to the flask and continue distilling until the distillate has no tract of cloudiness or a second phase as it drips from the condenser. Transfer the distillate to a separatory funnel, extract with a suitable solvent, and concentrate to a convenient volume. As the product is relatively volatile, do not try to evaporate to dryness if it can be avoided. The non-volatile residue in the distillation flask can also be recovered if necessary.

If it is desired to recover acids (e. g., phenols in the 4-AAP colorimetric method), make the solution acid before distilling; the distillate will then contain only neutral and acidic substances. The neutrals can then be separated during the extraction step. Basic materials remaining in the still pot, e. g., aniline, can then be recovered by making basic and distilling over a second fraction.

II SOLUBILITY SEPARATION OF EXTRACTS

A The organic contaminants from water as extracted from carbon or concentrated by liquid extraction ordinarily form an exceedingly complex mixture for which there is no one satisfactory separation procedure. If the analysis is directed towards a single component, the procedure may be designed for that purpose; otherwise a useful and generally applicable preliminary separation may be made on the basis of relative acidities.

B By extracting an ether solution of the sample with water, then with dilute hydrochloric acid, then sodium bicarbonate, and finally sodium hydroxide, a separation into water soluble, basic, strongly acidic, weakly acidic, and neutral fractions may be made. The portion insoluble in ether may also be recovered as an additional fraction. This method is obviously not suited to very volatile substances, nor to further separation of the water soluble fraction, nor to substances unstable in the presence of water or dilute acid or base; in addition, it must be remembered that substances whose partition coefficients are not extremely unfavorable to water may be present in several fractions in varying amounts.

C This procedure constitutes one of the best preliminary steps in analyzing any unknown sample and may often profitably be either preceded or followed by steam distillation (at various pH's) fractional crystallization, etc. Simple micro qualitative tests for nitroge., halogens, sulfur, phosphorus, etc., are also often useful; for a more extended discussion, see the texts mentioned in the bibliography.

D General

The sample should be substantially free of solvent. The amount may vary considerably but one-half gram is a very convenient amount; as little as 100 mg or even less may be used, but in this case there may be large percentage errors. Ethyl ether has proven to be an excellent solvent although often not all the sample will dissolve in it; benzene or chloroform may be used although these solvents are more likely to cause troublesome emulsions.

III LABORATORY DIRECTIONS

A Solubility Separation Procedure

Solution in Ether

- 1 Weigh previously dried sample contained in a tared 50 ml beaker (approximately 0.5 gm preferred). Also, weigh a 125 ml flask for the Water Solubles (WS).
- 2 Dissolve the sample with a small amount of methanol (about 1 ml). Add the methanol dropwise and stir with a rigid wire until the sample reaches a syrupy consistency.
- 3 Add 30 ml ether to the sample. Not all will dissolve. Collect the Ether Insolubles (EI) on a sintered glass funnel with suction and set aside for paragraph 4. Pour the contents of the suction flask into a 125 ml separatory funnel equipped with a teflon stopcock. (Stopcock grease will contaminate the sample.)

Ether Insolubles (EI)

- 4 Dissolve "EI" previously collected on the sintered glass funnel with methanol. Wash funnel once with a small volume of chloroform and collect in the suction flask. Transfer filtrate to the original tared beaker with methanol. Evaporate to dryness on a steam bath and record weight for Ether Insolubles.

Separation of the Water Solubles (WS)

- 5 Shake the ether solution ⁽³⁾ three times with 15 ml portions of distilled water. Drain the water layers into the weighed "WS" flask after each shaking. Evaporate the Water Soluble fraction to dryness on a steam bath with a jet of clean, dry air and record weight for Water Solubles.

Separation of Amine Fraction (B)

- 6 Mark flask "HCl". Shake ether solution three times with 15 ml portions of 5% HCl and drain aqueous layers into HCl flask after each shaking. Make this aqueous solution strongly basic by carefully adding NaOH pellets (about 30) or 25% NaOH. The solution becomes darker at this point. Set aside for back-extraction.

Separation of Strong Acid Fraction (SA)

- 7 Restore, original ether volume to 30 ml, if necessary. Mark flask "NaHCO₃". Shake three times with 15 ml portions of 5% NaHCO₃ and drain aqueous layers into NaHCO₃ flask after each shaking. Acidify aqueous solution in flask by adding sufficient concentrated HCl (dropwise because of liberation of CO₂) until strongly acid to litmus paper. About 4 ml is sufficient. The solution becomes cloudy at this point. Set aside for back-extraction.

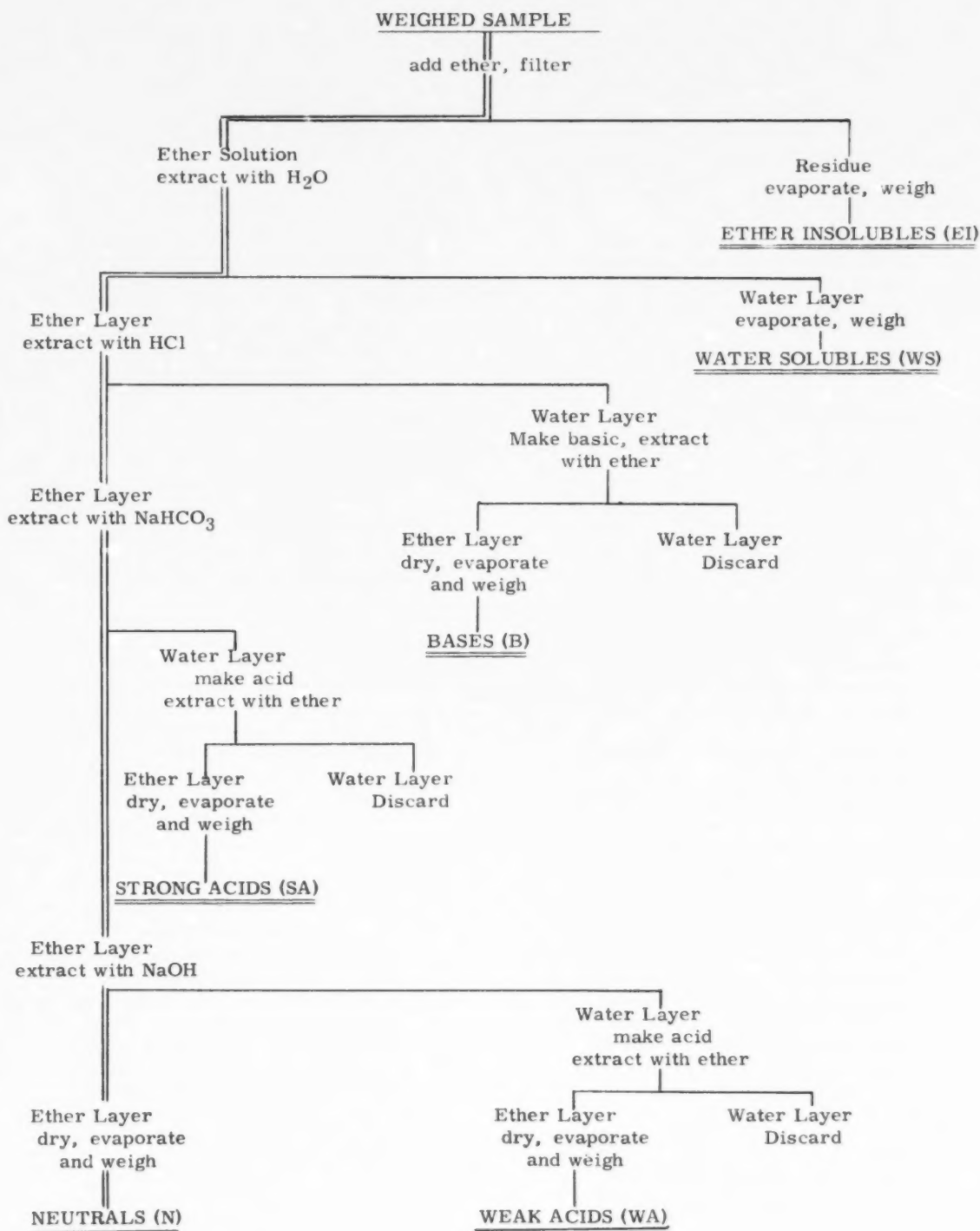
Separation of Weak Acid Fraction (WA)

- 8 Restore original ether volume to 30 ml, if necessary. Mark flask "NaOH". Shake three times with 15 ml portions of 5% NaOH and drain aqueous layers into "NaOH" flask after each shaking. Wash once with 15 ml distilled water and drain this into "NaOH" flask.

CAUTION: Tilt separatory funnel gently a few times, instead of shaking the first portion, as emulsions often occur.

If a small amount of the water wash remains emulsified, after draining, add several drops of saturated Na₂SO₄ solution and shake. This may help to break

Procedures for the Preliminary Separation of Extracts



the emulsion. Acidify aqueous solution in flask with concentrated HCl until strongly acid to litmus paper. About 8 ml sufficient. The solution becomes cloudy at this point. Set aside for back-extraction.

Neutral Fraction (N)

- 9 Mark flask "N". Pour the ether layer remaining in the separatory funnel into the flask "N". Add about 10 gm of anhydrous sodium sulfate, cap with aluminum foil and allow to stand overnight to dry the ether solution.

B Back Extraction

Strong Acids (SA)

- 10 Mark flask "SA". Transfer NaHCO_3 extract from step 7 to separatory funnel and shake several times, carefully, relieving pressure caused by evolution of CO_2 . Shake the NaHCO_3 extract, previously made acid, with a 15 ml portion of ether. Caution: Release CO_2 during first shaking repeatedly to avoid pressure build-up.

Drain the aqueous layer into the NaHCO_3 flask. Pour the ether layer into the "SA" flask. Return aqueous layer to separatory funnel and repeat extraction two more times with 15 ml portions of ether.

After all the ether layers have been collected in the "SA" flask, add about 10 gms anhydrous sodium sulfate, cap with foil and allow to stand overnight to dry the ether solution. Discard the aqueous NaHCO_3 portion which should be almost colorless.

Weak Acids (WA)

- 11 Mark flask "WA". Shake the NaOH extract, previously made acid, three times with 15 ml portions of

ether and collect in flask "WA" as in step 10. Add sodium sulfate to dry, cap with foil and allow to stand. Discard aqueous NaOH portion which should be almost colorless.

Bases (B)

- 12 Mark flask "B". Shake the HCl extract, previously made basic, three times with 15 ml portions of ether and collect in flask "B". Add sodium sulfate to dry, cap with foil and allow to stand. Discard aqueous HCl portion.

C Transfer

- 13 Weigh four flasks and mark them "N", "WA", "SA", and "B". Record weights of each. Transfer the corresponding dried ether solutions from the Na_2SO_4 into the weighed flasks by filtering through filter paper.
- 14 Evaporate the ether from the above fractions with the aid of clean, dry circulating air and gentle heat on a steam bath. The ether solutions should be removed from the air and heat before totally dry and allowed to dry spontaneously to prevent loss of volatile components. Record the weight of the dried fractions.

D Chromatographic Separation

- 15 Weigh a 10 ml beaker and record weight. Dissolve the Neutrals in about 5 ml anhydrous ether, transfer most of the Neutrals to the beaker and place the remaining few drops in a vial for infrared analysis. Wash the (N) flask with ether and pour washings into vial also. Dry the Neutrals contained in the beaker by spontaneous evaporation and record weight.

- 16 Weigh a set of 125 ml Erlenmeyer flasks and mark them Aliphatics, Aromatics and Oxys. Record weights of each.
- 17 Fill the chromatographic column with 4 1/2" of silica gel and tap down to about 4". Place the Aliphatics flask under the column. Add about 20 ml of iso-octane to wet the column.

Aliphatics

- 18 Before the iso-octane reaches the level of the silica gel, add the Neutrals which have been previously adsorbed onto a small amount of silica gel by stirring in the beaker with a rigid wire. Elute with 85 ml iso-octane. Rinse the beaker with a small portion and add to the column first. Allow this volume to enter silica gel before adding the remainder of the iso-octane. Collect the eluent in the Aliphatics flask, evaporate to dryness with the aid of gentle heat and circulating air.

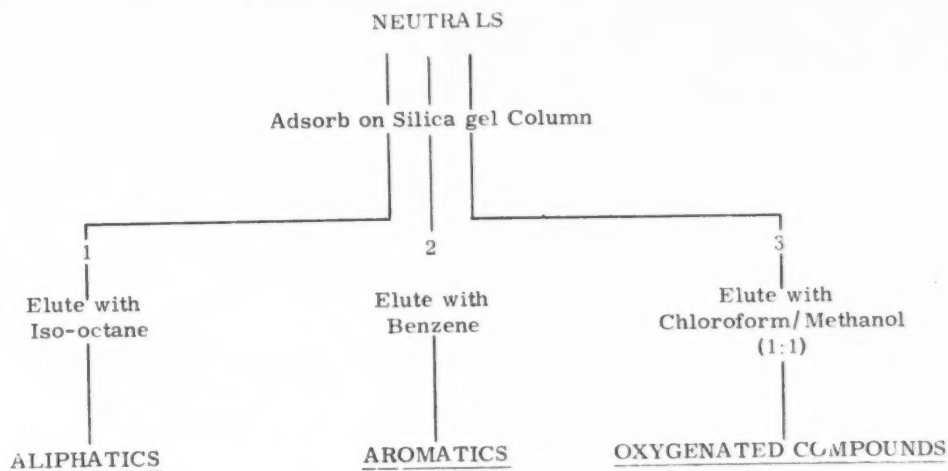
Aromatics

- 19 Place the Aromatics flask under the column. Elute column with 85 ml of Benzene-rinsing the beaker with a small portion and adding immediately as the previous eluent reaches the level of the silica gel. Allow this volume to enter silica gel before adding remainder of benzene. Evaporate with the aid of gentle heat and circulating air. The Aromatics fraction should be removed from the air and heat before totally dry and allowed to dry spontaneously to prevent loss of volatile components.

Oxys

- 20 Place the Oxys flask under the column. Elute with 85 ml of a 1:1 methanol chloroform mixture - rinsing the beaker with a small portion and proceed as before. Evaporate to dryness with the aid of gentle heat and circulating air.

CHROMATOGRAPHIC SEPARATION OF NEUTRALS



- 21 Record weights of the dried Aliphatics, Aromatics and Oxys.

NOTE: (All chromatographic solvents should be re-distilled before use)

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These books deal principally with chemical methods of identification; Shriner, Fuson and Curtin and Cheronis and Entrikin are recommended for those new to the field. For instrumental analysis and quantitative work, see standard works in those fields.

This outline was prepared by R. H. Burtschell, Chemist, formerly with AWTL, NERC, Cincinnati, OH and J. J. Lichtenberg, Pesticides Identification Group Leader, AQCL, NERC, EPA, Cincinnati, OH 45268.

PESTICIDES IN WATER

I INTRODUCTION

- A The contamination of the environment with persistent pesticides has become increasingly more apparent as a health hazard to the general population.
- B Because of the health implications of this environmental contamination, a report was prepared by the President's Science Advisory Committee on the use of pesticides.
- C The use of pesticides has increased our material standard of living during the 20th Century by increased pest control.
- D The discovery, manufacture and application of new pesticide chemicals has benefited agriculture and consequently food-stuffs of the highest quality are currently available.
- E Besides enabling spectacular increases in agricultural production pesticides have freed man from communicable diseases to an unprecedented extent.
- F Pesticides are now detectable in many food items, in some clothing, in man and animals and in various parts of our natural surroundings.
- G Carried from one locality to another by air currents, water runoff, or living organisms (either directly or indirectly through extended food chains), pesticides have traveled great distances and some of them have persisted for long periods of time.
- H Since pesticide chemicals are designed to kill or metabolically upset some living target organism, they are potentially dangerous to other living organisms. Most of them are highly toxic in concentrated amounts, and in unfortunate instances they have caused illness and death of people and wildlife.
- I The most popular group of pesticidal agents, the chlorinated hydrocarbons, will with a few exceptions, accumulate in the body fat of man and animals.

- J The end result of such accumulation on the well being of man and animals is not yet known.

II NATURE OF PESTICIDES

A Classification

Pesticides can be classified according to their use as insecticides, herbicides, rodenticides, miticides, nematocides, and fungicides. They can also be classified according to their origin.

1 Mineral origin (inorganic)

These include arsenicals and inorganic compounds containing sulfur, copper and fluorine.

2 Botanical origin (natural organic)

These include nicotine, pyrethrum and red quill.

3 Synthetic origin (organic)

a Insecticides

1) Chlorinated hydrocarbons

These compounds all contain chlorine, hydrogen and carbon (see Figure 1). A few such as dieldrin and endrin also contain oxygen. Otherwise, they vary widely in their chemical structure and activity. They affect the central nervous system; however, the basic mode of toxic action is not known for any of them.

2) Organic phosphorus compounds

These compounds are derivatives of phosphoric acid (see Figure 2) and are characterized by similar structure. Their mode of toxic action is to interfere with cholinesterase enzyme activity.

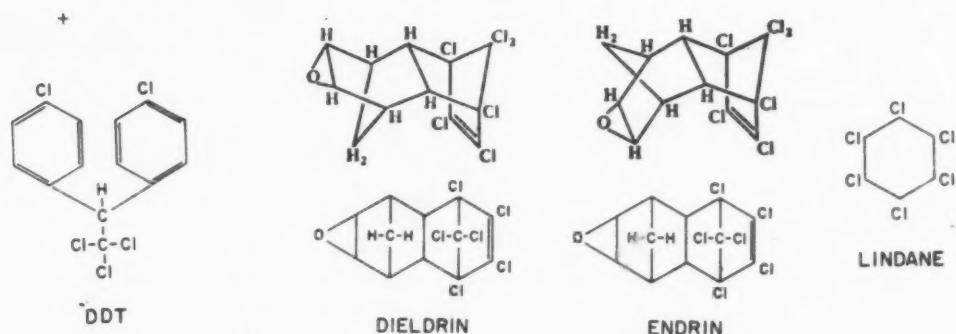


Figure 1. CHLORINATED HYDROCARBON PESTICIDES

b Phenoxyalkanoic acid herbicides

These compounds are similar to chlorinated hydrocarbons; however, they are classified separately because they also contain free carboxyl groups. Examples of such compounds are 2, 4D, 2, 4, 5-T and Silvex.

c Miscellaneous compounds (e. g., carbamates and dithiocarbamates)

B History of Use and Control

- 1 In 1000 B. C., Homer first used sulfur as a fumigant for pest control.
- 2 The insecticidal properties of DDT were discovered in 1939.
- 3 Organic phosphorus compounds first appeared on the market in 1945.
- 4 The production of synthetic organic pesticides has skyrocketed from 0 lbs. in 1942 to 763 million lbs. in 1965. A ten-fold increase in pesticide output is predicted in the next 20 years.
- 5 This large production output of toxic compounds has necessitated governmental programs of control.
 - a The original programs were designed to evaluate mostly short-term effects (acute toxicity).

1) 1947 - The Federal Insecticide, Fungicide and Rodenticide Act required proper labeling of all formulations containing pesticides.

2) 1954 - The Miller Amendment to the Federal Food, Drug and Cosmetic Act of 1938 required that tolerances be established for pesticide residues on crops.

b In recent years, concern has arisen over long-term effects and the biological magnification of pesticides in the environment.

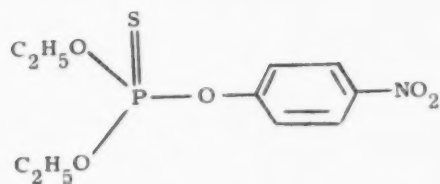
C Gains from Use

- 1 Promotion of health
 - a Directly through the control of vector-borne diseases (e. g., malaria).
 - b Indirectly through increased and improved agricultural production.
- 2 Elimination of nuisances.

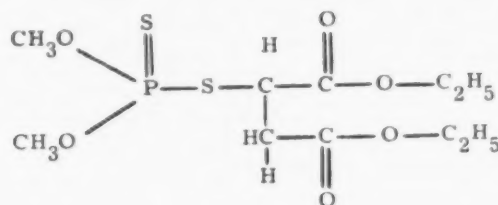
III PESTICIDES AS A WATER POLLUTION PROBLEM

A History

- 1 The first awareness that synthetic pesticides could be a pollution problem



PARATHION



MALATHION

Figure 2. ORGANIC PHOSPHORUS PESTICIDES

in water occurred in 1950 when extensive fish kills happened in 14 streams tributary to the Tennessee River in Alabama. These kills were caused by insecticides washed from cotton fields. (2)

- 2 In 1954, Tarzwell and Henderson confirmed that insecticides could run off soil with rainwater. They did this by applying clay granules containing 11% dieldrin to a measured grassy sod slope at a rate of 4.66 lbs. per acre and then assaying the dieldrin recovered in the runoff. (2)
- 3 In 1959, the PHS established a Pesticide Pollution Project under the direction of H. Page Nicholson in Atlanta, Georgia. These activities are now located at the Southeast Water Laboratory, Athens, Georgia.
- 4 Fish kills occurring in the lower Mississippi in the winters of 1960, 1961, 1962 and 1963 have been attributed to synthetic pesticide pollution. Less than one $\mu\text{g/l}$ (water concentration) of endrin was responsible for the kills.

B Mode of Entrance into Water

- 1 Pesticides may be directly applied to water supplies for one of the following purposes.
 - a To control aquatic insects
 - b To control algal growths
 - c To poison fish in order to permit restocking with desired varieties.

2 Industries may discharge liquid wastes containing pesticides.

3 Runoff of pesticides from agricultural lands and forests is dependent on several factors.

- a Solubility of the pesticide in water
- b Persistence of the pesticide in the soil which in turn is dependent on the pH and temperature of the soil
- c Quantity of pesticide applied to the soil
- d Formulation of the pesticide
- e Method of application of pesticide
- f Slope of the land
- g Soil characteristics
- h Volume and intensity of rainfall
- i Soil conservation practices

C Effects

1 Acute toxicity

- a An estimated 150 human fatalities occur annually in the United States from pesticides. (Note: this is about the same death rate as that due to aspirin).
- b Bioassay studies⁽⁴⁾ confirm that many fish are very sensitive to synthetic pesticides (Table 1).

Table 1. COMPARATIVE TOXICITY OF ORGANIC PHOSPHORUS
AND CHLORINATED HYDROCARBON INSECTICIDES TO
BLUEGILLS IN SOFT WATER

Organic phosphorus insecticide	96-hour TL _m (mg/l)	Chlorinated hydrocarbon insecticide	96-hour TL _m (mg/l)
Gluthion	0.0052	Endrin	0.0006
Malathion	0.090	Toxaphene	0.0035
Parathion	0.095	Dieldrin	0.0035
TEPP	1.1	DDT	0.016
Methyl		Heptachlor	0.019
Parathion	1.9	Lindane	0.077
OMPA	110		

Note: Above tests were run under standardized conditions with soft water as a diluent at 25°C.

2 Chronic toxicity

- a In recent years greater concern has arisen over the possible long term effects of the accumulation of small amounts of pesticides in the human body.
- b Another aspect of this problem is the possibility of synergistic effects being exerted as these pesticides accumulate.

3 Taste and odor

Pesticides and the solvents used in pesticide formulation can be highly odorous.

in water in the microgram and nanogram per liter ranges. Procedures being developed are according to the following general lines.

a Chlorinated hydrocarbons⁽¹⁾

- 1) Recovery of pesticides from water using the carbon filter or liquid-liquid extraction.
- 2) Separation and identification of extracts.
 - a) Adsorption chromatography serves both as a separation and clean-up procedure.
 - b) Thin layer chromatography serves both as a clean-up technique and as a means for confirmatory identification.
 - c) Gas chromatography can be used for identification and quantitative assay of pesticides.
 - d) Paper chromatography can also be used for confirmatory identification.

IV SOLUTION TO THE PROBLEM

A More research is needed in order to answer adequately the following questions:

- 1 How general is the occurrence of pesticides in surface and ground waters? In order to answer this question, it is necessary that techniques of analysis be able to measure pesticides

3) Infrared identification

Absolute identifications of pesticides can be made using micro infrared spectroscopy. However, it is necessary to have recovered 1-10 micrograms of pesticide when using the Perkin Elmer Model 421 instrument with 6X beam condenser and a 1.5 mm KBr disc.

b Organic phosphorus

- 1) It is possible to measure organic phosphorus compounds using electron capture gas chromatography.
- 2) However, the most popular techniques developed so far have been biochemical assays which measure inhibition of the enzyme cholinesterase.⁽⁶⁾
- 2 What are the less obvious effects of pesticides on organisms in the aquatic environment?
- 3 What factors relate to the presence and persistence of pesticides in water?
- 4 How well are pesticides removed in water treatment?⁽⁶⁾

B More Judicious Use of Pesticides

As a result of the special White House Report (May 15, 1963) and recent Senate hearings, the Department of Agriculture has made changes in some of its big spray projects. Mirex, a compound with low toxicity to fish, was chosen to replace heptachlor in the Southeast fire ant program. Sevin, also a compound with low toxicity to fish, was chosen to replace DDT to control the hemlock looper in Washington State.

V SUMMARY

The nature of pesticides in general is presented and their potential as a water pollution problem is discussed.

ACKNOWLEDGEMENT:

Certain portions of this outline contains training material from a prior outline by P. F. Hallbach.

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This outline was prepared by Betty Ann Punghorst, Chemist, formerly with National Training Center, MDS, WPO, EPA, Cincinnati, OH 45268.

INTRODUCTION TO PESTICIDE RESIDUE ANALYSIS

I INTRODUCTION

- A Because of the complexity of compounds which may be present in any one sample, it is necessary to have available analytical procedures that will enable the chemist or technician to determine the concentration of a large number of pesticides by a single procedure.
- B The size of the sample used for analysis is dependent on the nature of the sample and the contamination present.
- C Most pesticides are soluble in polar and nonpolar organic solvents, and their separation can be easily affected by their extraction into an organic solvent.
- D The first step in residue analysis is to isolate and concentrate pesticide residues by extraction into a suitable solvent such as hexane or petroleum ether.
- E Many compounds, such as oils, fats, lipids, and plant pigments are also soluble in organic solvents. In most cases they must be removed before the pesticide residues can be effectively analyzed.
- F The process for their removal is referred to as cleanup and is usually accomplished by one or more of the following three procedures:
 - 1 Cleanup by partition
 - 2 Cleanup by sorption
 - 3 Cleanup by saponification

II SAMPLE ANALYSIS

- A Many analytical procedures are available for the analysis of pesticide compounds.
- B The complexity of the number of compounds which can be present in a water sample precludes the use of an individual technique for each specific compound.

C Pesticide residue analysis is generally accomplished by the use of some form of chromatography.

D This technique has become widely used for pesticide residue analysis within the last ten years, and it is used for the separation of complex mixtures of compounds.

E Three types of chromatography can be used for the quantitative estimation of pesticide residues. They are:

- 1 Paper chromatography
- 2 Thin-layer chromatography
- 3 Gas chromatography

F Methods of Analysis

1 Paper chromatography

- a Based on the principle of liquid-liquid partition between a mobile and immobile solvent supported by a sheet of filter paper.
- b This procedure is referred to as qualitative and semi-quantitative.
- c Sensitivity for most chlorinated hydrocarbon residues is equivalent to about 0.05 ppm.
- d An extensive "cleanup" is required to remove interfering materials.
- e About two days are required for a single analysis. Several samples, however, may be analyzed over this period of time.
- f Investigations are now in progress with respect to modification of the supporting material to allow the

use of this method for the detection of organic phosphate residues.

2 Thin layer chromatography

- a Similar to paper chromatography. Technique is based on adsorption rather than on partition. It is based on the differential migration of pesticides through a solid adsorbent by the capillary attraction of the adsorbent for an organic solvent.
- b Glass plates are used which are coated with an adsorbent.
- c The chromatogram is "developed" with suitable organic solvents.
- d Technique has many advantages over paper chromatography.

3 Instrumental

a Gas chromatography

- 1) Based on the principle of separation by distribution between two phases, liquid and gas. Separation depends on the relative affinity of the immobile phase for the various pesticides.
- 2) Utility value of this equipment depends upon the type of detector employed.
 - a) The thermal conductivity detector is not sufficiently sensitive for residue analysis.
 - b) Ionization detectors are extremely sensitive but require too much "cleanup" of food materials to be of practical use in the determination of chlorinated hydrocarbons. These detectors employ changes in the electrical conductance of the effluent gas brought about by various methods of ionization.

- c) Electron capture detector responds only to compounds containing atoms of high electronegativity. Thus small amounts of halogenated compound can be detected in the presence of relatively large amounts of compounds containing only carbon and hydrogen. This detector has been used to detect nanogram concentrations (10⁻⁹g) of the halogenated hydrocarbons in the hexane extract of spiked food samples.

b Gas chromatography - microcoulometric analysis

- 1) This is a highly integrated instrument functioning via gas chromatographic separation, combustion, electrometric titration, and electronic recording of results.
- 2) Chlorinated hydrocarbon pesticides may be determined by direct titration of the Cl ion. Phosphates do not appear to interfere.
- 3) Pesticides containing a sulfur atom, may be determined by reducing the sulfur to hydrogen sulfide which is titrated with the silver ion.
- 4) At the present time a rather extensive "cleanup" is required for analyses of milk and milk products.
- 5) Results to date, indicate a sensitivity of about 0.01 ppm.

c Infrared spectroscopy

- 1) This procedure has been applied to foods other than milk for the detection of organic phosphate residues, by using the "fingerprint" method.

- 2) Instruments available today lack the sensitivity required for residue analyses in milk.
- 3) An extensive pre-analysis "cleanup" procedure is required.
- 4) Infrared spectrophotometers are expensive instruments requiring well trained analysts for their use.

d Gas chromatography - infrared spectroscopy

- 1) Combines the chromatographic separation of pesticides and the "fingerprint" identification of infrared spectroscopy.
- 2) Falls heir to the same disadvantages as the infrared spectrophotometer alone, except that the "cleanup" procedure is not as extensive.

4 Chemical

a Colorimetric

- 1) Methods are based on the formation of a colored compound from the pesticide or a decomposition product of the pesticide.
- 2) Such methods generally approach the sensitivities required for residues in milk and milk products.
- 3) All procedures in this group are time consuming.
- 4) While these methods are often referred to as being specific for a given pesticide, almost all of them experience some interference from chemically related compounds.

- 5) Chlorinated hydrocarbon, organic phosphate, and carbamate residues may be determined by chemical-colorimetric procedures.
- 6) Expensive special laboratory equipment is usually not necessary.

b Titrametric

1) Total chlorine procedures

- a) Analyses are extremely time consuming.
- b) Results are general.
- c) Procedures are sensitive to very low residue concentrations.
- d) Equipment requirements are essentially that of an operating chemical laboratory.
- e) Blank values are often quite variable.

- 2) These procedures are based on the chemical separation of pesticides or decomposition products thereof and estimation by titration with standard solutions.

III THE FUTURE OF THE RESIDUE PROBLEM

A Agents to Use

B Public Relations

C Analytical Methods

D Tolerances

Introduction to Pesticide Residue Analysis

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This outline was prepared by Paul F. Hallbach,
Chemist, National Training Center,
MDS, WPO, EPA, Cincinnati, OH 45268.

INTRODUCTION TO GAS-LIQUID CHROMATOGRAPHY

I INTRODUCTION

A Definition

Gas-liquid chromatography is an analytical method for the separation and identification of a mixture of volatile (usually organic) components in a sample. As with any chromatographic technique the column consists of two phases, the immobile or stationary phase (a liquid on an inert solid support), and the mobile phase (an inert gas). The column functions to separate the sample components because they have varying vapor pressures and affinities for the stationary phase. In many ways the column behavior resembles that of fractional distillation. The partition which occurs between the mobile and immobile phases will thus cause the components to proceed through the column at varying rates. The separation is recorded and quantitated by the detector system.

B Advantages

- 1 GLC can be used to separate compounds of similar boiling points which cannot easily be separated by distillation. (See Table 1)
- 2 GLC can be extremely sensitive; for example, using the electron capture detector it is possible to "see" picogram (10^{-12}) quantities.

Table 1. SEPARATIONS BY GLC

Compounds	Reference
3-Methylcyclohexene (B.P. 104°C) and 4-Methylcyclohexane (B.P. 103°C)	Aerograph Research Notes (Spring 1964)
Cyclohexane (B.P. 80.8°C) and Benzene (B.P. 80.2°C)	Chromosorb Newsletter (FF-104)

C Disadvantages

- 1 Due to the extreme sensitivity possible it is often necessary to apply extensive cleanup techniques.
- 2 The many variables of the technique require a skilled analyst.

II COMPONENTS OF A GAS CHROMATOGRAPH (See Figure 1)

A Gas Supply

The mobile phase (carrier gas) transports the sample components through the column into the detector. The type of gas used varies with the detector. (See Table 2)

B Injector

Liquid samples are manually introduced into the heated injector block through a rubber septum by means of a syringe. Automatic liquid injectors as well as injection systems for solid and gaseous samples are commercially available.

C Column

The vaporized sample enters the column which can be glass or metal and of varying length (1' - 20') and diameter (1/8" - 1/4"). The column is packed with the stationary (immobile) phase and contained within a constant temperature oven.

1 Solid support

The solid support should have a large surface area yet be inert so that active sites will not cause adsorption of sample components. Diatomaceous earths, teflon and glass beads have been used. (See Table 3)

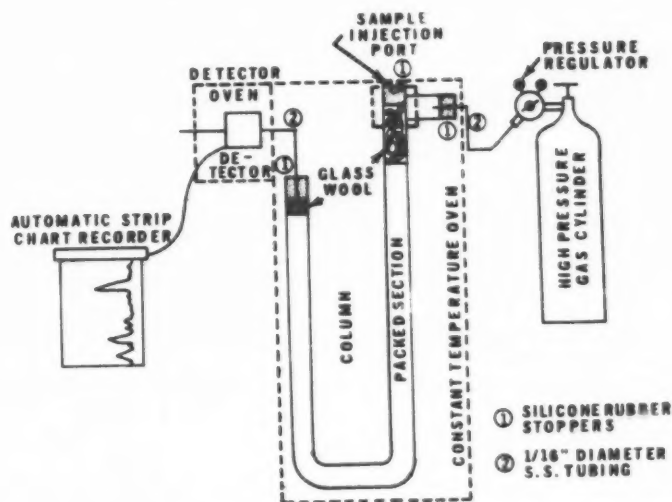


Figure 1. COMPONENTS OF A GAS CHROMATOGRAPH*

Table 2. CARRIER GASES

Detector	Carrier gas
Thermal conductivity	Helium (Purified, Grade A)
Microcoulometric	Helium (Purified, Grade A)
Flame ionization	Hydrogen (Purified)
Electron capture	Nitrogen or a mixture of 95% argon and 5% methane (Purified)

Table 3. SOLID SUPPORTS

Support	Surface area (m ² /gm)
Chromosorb P (Diatomaceous Earth)	4.8
Chromosorb W (Diatomaceous Earth)	1.2
Chromosorb G (Diatomaceous Earth)	0.5
Chromosorb T (Teflon)	7.0 - 8.0

2 Stationary liquid

The separation and partition occurring in the column is directly affected by the choice of stationary liquid. For example, in the separation of benzene (B. P. 80.1°C) and cyclohexane (B. P. 80.8°C), the choice of a non-polar phase such as hexadecane results in benzene preceding cyclohexane off the column. However, if a more polar phase such as benzylbiphenyl is chosen cyclohexane precedes benzene. Table 4 shows some typical stationary liquids and their uses. (NOTE: One requirement for any liquid is that it have a high boiling point so that it will not boil off the column)

D Detector

The detector or brain of the gas chromatograph senses and measures the quantity of sample component coming off the column. The detector should be maintained at a temperature higher than the column so that condensation does not occur in the detector block. Several types of detectors are in use today.

*Reproduced (with permission) from Chemistry. (37:11, p. 13, November 1964).

Table 4. STATIONARY LIQUIDS

Stationary liquid	Used to separate
Silicone oils QF-1, Dow Corning 200, and Dow 11, OV-1, OV-3, OV-17, OV-Z10, OV-225	Chlorinated hydrocarbons pesticides
Silicone oil SE-30	Homologous series of n-alkanes
Benzyl-Cyanide-Silver Nitrate	Homologous series of olefins
Polyethylene Glycol	Amines
Cyano Silicone	Steroids

1 Thermal conductivity

This detector consists of a Wheatstone bridge two arms of which are thermal conductivity cells each containing a small heated element. When only carrier gas is flowing through both the sample cell and reference cell, the resistance of the heated element is constant in both cells. The bridge remains balanced and baseline is recorded. However, when carrier gas plus sample component enter the sample cell, the thermal conductivity in that cell changes thus also producing a change in the resistance of the heated element. The bridge becomes unbalanced and a peak is recorded. The main disadvantage of the TC cell in water pollution work is its lack of sensitivity.

2 Ionization detectors

a Flame

This detector consists of a flame situated between a cathode and anode. As carrier gas alone burns, some electrons and negative ions are produced which are collected at the anode and recorded as baseline. When carrier gas plus sample component are burned, more electrons and negative ions are produced which result in a peak on the recorder. The detector is capable of "seeing" nanogram quantities of organic compounds; however, the detector is sensitive to all organic compounds. This lack of specificity produces disadvantages in the analysis of water

extracts which contain a variety of naturally occurring organics.

b Electron capture (See Figure 2)

This detector consists of a radiation source (e.g., tritium) capable of producing slow electrons in a carrier gas such as nitrogen. The electrons collected at the anode are recorded as baseline. When sample components which have an electron affinity (e.g., chlorinated hydrocarbons) enter the detector, electrons are "captured." The subsequent decrease in current is recorded as a peak. The detector has the advantage that it is extremely sensitive (pico-gram range) and is somewhat selective.

c Thermionic

A recent adaptation of the flame ionization detector shows promise for the specific analysis of organic phosphorus compounds. An alkali salt is incorporated into the design of a conventional flame ionization detector so that the salt heated by the flame produces an ion current. When compounds containing phosphorus emerge from the column, they give 600X the response with this detector as with the conventional flame.

3 Microcoulometric

Although less sensitive (by approximately a factor of 10) than electron capture,

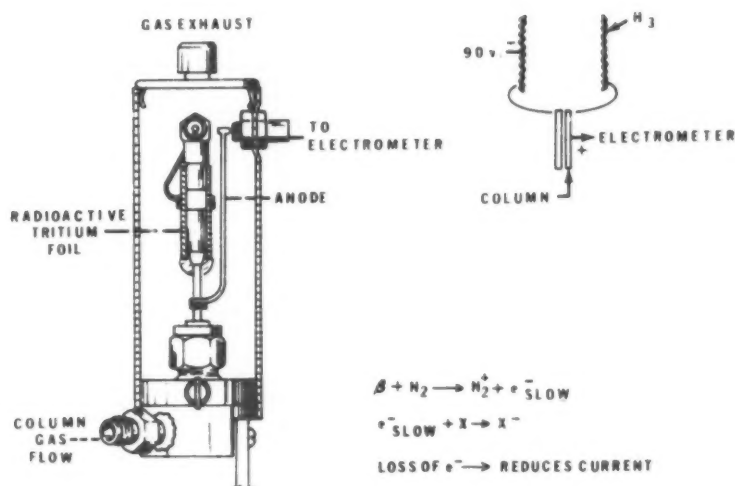


Figure 2. ELECTRON CAPTURE DETECTOR (Wilkins Instrument Company)

this detector is finding wide use in pesticide analysis. This highly specific detector consists of titration cells for the measurement of chloride-containing and sulfur-containing compounds. The sample component emerging from the column is combusted to produce HCl or SO₂, respectively. HCl is continuously titrated by silver ions present in the cell; the amount of current required to regenerate these silver ions is recorded as a peak. The system for sulfur containing compounds is analogous except that SO₂ produced is continuously titrated by I₂ which is subsequently regenerated.

Another microcoulometric detector has recently been applied to the specific determination of nitrogen. It is based on the reduction of nitrogen-containing compounds to NH₃ which is then titrated by H⁺ in the titration cell.

4 Coulson Electrolytic Conductivity

This detector was primarily developed for the detection of organic halides, organic nitrogen compounds, and organic sulfur compounds.

The unit consists of pyrolyzer with a separately heated inlet block, water circulating and purification system, detector cell and dc conductivity bridge. The sample is oxidized or reduced and reaction products form electrolytes when dissolved in the deionized water. Changes in conductivity between two platinum electrodes are measured by the dc bridge. (Figure 2 a)

E Recorder

The recorder system registers the response of the detector to sample components. In the case of ionization detectors, it is often necessary to employ an electrometer in order to amplify the small current changes.

Expensive integration and digital read-out equipment is also available to facilitate measurement of peak areas.

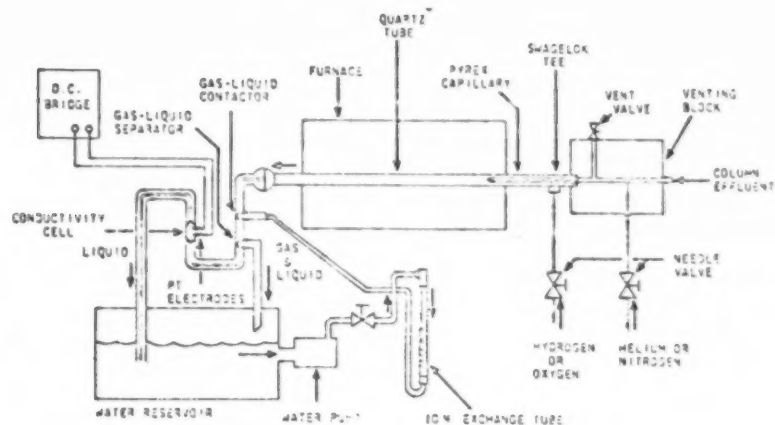


Figure 2 a. FLOW DIAGRAM OF ELECTROLYTIC CONDUCTIVITY DETECTOR
(Coulson Instruments Company)

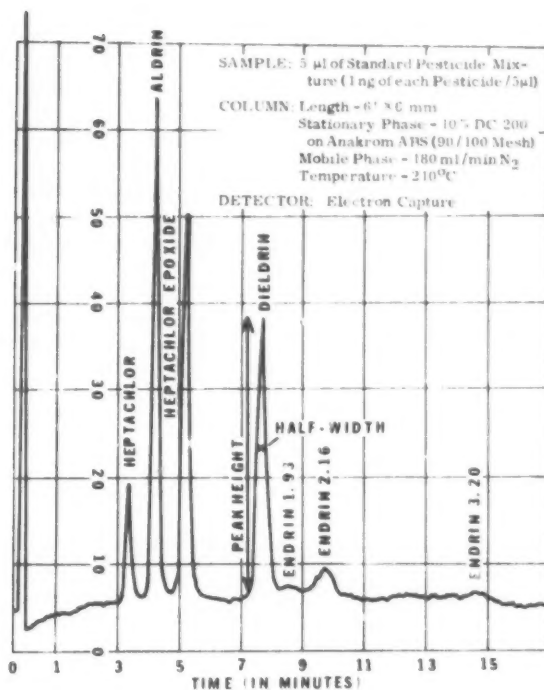


Figure 3. GAS CHROMATOGRAM OF PESTICIDE MIXTURE

III QUALITATIVE ANALYSIS

A Retention Time

The retention time of a sample component is defined as the time it takes for that component to travel through the column. There are a number of variables which affect the retention time of a compound.

1 Physical parameters of column operation

- a Column length
- b Column temperature
- c Carrier gas flow rate

2 The nature and amount of stationary liquid itself

For a given set of column conditions, a specific compound will have a specific retention time (See Figure 3 and Table 5). Various column and detector combinations can be used to confirm identification.

B Retention Volume

Retention volume is defined as the total volume of gas required to move a component through the column.

$$\text{RETENTION VOLUME (R}_V\text{)} = \frac{\text{RETENTION TIME (R}_T\text{)}}{\text{FLOW RATE}} \times \text{FLOW RATE}$$

C Relative Retention Times and Volumes

It is possible to interpret data more easily by reporting retention data relative to a particular compound (e.g., aldrin as in Table 5).

IV QUANTITATIVE ANALYSIS

A Measurement of Peak Area

The quantity of sample component present is directly proportional to the area under its peak. (NOTE: This assumption can only be made if it has been previously determined that a linear response is obtained in the range under study.) The following are a few of the ways in which this area can be measured.

- 1 Planimeter
- 2 Triangulation
- 3 Peak height $\times$ half-width (see dieldrin peak in Figure 3)

$$\text{AREA} = \text{peak height} \times \text{peak half-width}$$

4 D i c integrator

B Measurement of Peak Height

With the electron capture detector it may be possible to use peak height for quantitative measurements where the following conditions are met.

Table 5. RETENTION DATA FOR FIGURE 3

Pesticide	Retention Time (R _T)	Relative Retention Time
Heptachlor	3.3 minutes	0.79
Aldrin	4.2	1.00
Heptachlor Epoxide	5.3	1.26
Dieldrin	7.7	1.84

- 1 A steady baseline is obtained.
- 2 Retention times can be reproduced from one injection to the next.

V SUMMARY

The basic components of a gas chromatograph have been described. Elementary aspects of quantitative and qualitative analysis are presented.

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This outline was prepared by B. A. Punghorst, Chemist, formerly with National Training Center, MDS, WPQ EPA, Cincinnati, OH 45268.

THIN-LAYER CHROMATOGRAPHY

I INTRODUCTION

A Historical

- 1 Adsorption chromatography was discovered by Tswett in 1903. Tswett found that plant pigments (e. g., chlorophylls) can be separated by filtering a solution of them in petroleum ether through a column of calcium carbonate. He noticed that yellow and green zones were formed in the column.
- 2 Adsorption chromatography was introduced in 1931 when the method was used for the preparative chemistry of the polyene pigments, for example the separation of carotene into its components.
- 3 Partition chromatography for hydrophilic compounds was introduced in 1941.
- 4 Thin-layer chromatography was introduced by Stahl in 1956. He described an ingenious and practicable device for preparing layers of silica gel about 250 microns thick with a plaster of paris binder.
- 5 Thin-layer chromatography has proven useful for the quantitative estimation of pesticides and for the "clean-up" of sample aliquots prior to the estimation of their components by gas chromatography.
- 6 Quantitative thin-layer chromatography is used to measure the amount of the components present. This is done by estimating the density of the spots as compared to standard spots.
- 3 When the stationary phase is a simple liquid or a liquid held on some type of support, the chromatography is considered to be partition chromatography.
- 4 Diagnostic or qualitative thin-layer chromatography is used to determine the number of components in a system.
- 5 Preparative thin-layer chromatography is used to separate a mixture into its components in such a way that reasonable amounts can be isolated and studied.

B Theory

- 1 Thin-layer chromatography is an analytical technique which has proven useful for the separation of components in a given system and to evaluate the components in that system.
- 2 All of the techniques of chromatography are based upon the same simple principle. They involve a moving system of some type (liquid or gas) which is in

equilibrium with a stationary phase. When the stationary phase is a solid and the forces acting between it and the mixture are adsorptive in nature, the technique is called adsorption chromatography.

II DEFINITION OF TERMS

- A Adsorbent. The finely divided powder which makes up the stationary phase in adsorption chromatography, or holds the stationary liquid in partition chromatography.
- B Layer. The thin layer of adsorbent bound or unbound, deposited on a glass plate.
- C Spotting. The application of the substance to the thin layer adsorbent.
- D Development. The passing of a liquid through the layer to effect a separation.
- E Developer. The liquid used for the development.
- F Eluent. The complete removal of a substance from the adsorbent layer.

G Visualization. The rendering visible of the results of a developed chromatogram.

H Ratio factor " R_f ". The distance traveled by a given substance divided by the distance traveled by the solvent front. Both measured as the origin.

III ADSORBENTS

A Silicic Acid or Silica Gel

Chemically identical, but the gel is prepared in such a manner as to enhance its adsorptive power.

B Aluminum Oxide or Alumina

C Diatomaceous Earth

D Powdered Cellulose

E Essentially any substance which has desirable adsorptive and chemical characteristics can be successfully used in thin-layer chromatography.

IV PREPARATION OF PLATES

A Methods

- 1 Spreading or spraying of slurries
- 2 Dipping in a slurry

B Equipment

- 1 Kirchner type
- 2 Stahl type
- 3 Microchromatoplates

C Layer thickness

- 1 For qualitative analysis thin layers (250 microns) are used.
- 2 For preparative thin layer thicker layers may be used.

D Moisture Content

- 1 Trace quantities of water greatly reduce the activity of various adsorbents.
- 2 Normally plates are activated by drying at 110°C for one hour and then stored in a desiccator.

V TECHNIQUE

A Spotting the Plate

- 1 The activity of the layer is affected by humidity and temperature.
- 2 The sample is applied so that the direction of the development is opposite to the direction in which the layer has been applied.
- 3 Use of spotting template
- 4 Use of micro-pipette and micro-liter syringe
- 5 Size of sample spots
- 6 Use of standards

B Solvent System

- 1 Investigation of single and multi-component systems
- 2 Carbon tetrachloride, hexane, and n-heptane as solvents for separation of pesticides

C Development of the Plate

- 1 Development tank
- 2 The plate is placed in a glass tank containing the solvent. When the solvent front reaches the upper reference line (10 cm) the plate is removed and the solvent is allowed to evaporate.

VI VISUALIZATION

A Rhodamine B

- 1 Spray reagents - Rhodamine B, spirit soluble, 0.1 mg per ml in ethanol
- 2 Method of spraying
- 3 Advantages of Rhodamine B
 - a The pesticide is not destroyed.
 - b The exact position of the pesticide can be determined.
 - c The use of a selective solvent permits the pesticide to be eluted from the adsorbent while the Rhodamine B is retained.

B Impregnation of Plates with AgNO_3

- 1 Increased sensitivity for quantitative estimation
- 2 Elimination of spraying

C Exposure to UV Light

- 1 Pesticides appear as quenched areas in the fluorescent background.

- 2 Recovery studies using Rhodamine B spray are comparable to those using silver nitrate spray. The following pesticides may be seen at the 10 μg level under UV light after Rhodamine B spray:

endrin	heptachlor
dieldrin	heptachlor epoxide
DDT	methoxychlor
aldrin	toxaphene
lindane	DDE
chlordan	ovex
parathion	tedion
methyl parathion	

- D Suggested systems for adsorption chromatography are given in Table 1.

E Recovery

- 1 Elution of pesticides from the thin layer with a mixture of ethyl and petroleum ethers.
- 2 Recovery zones as shown in Figure 1.
- 3 Silica gel collection assembly - Figure 2.

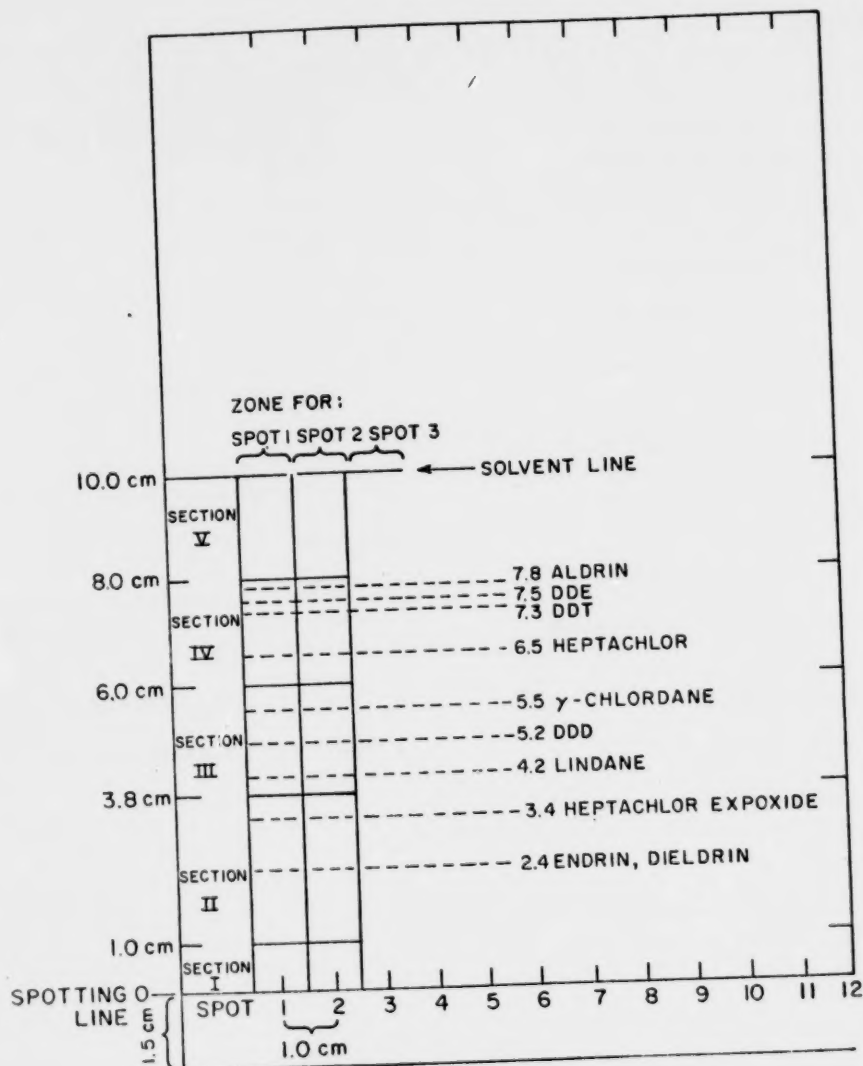
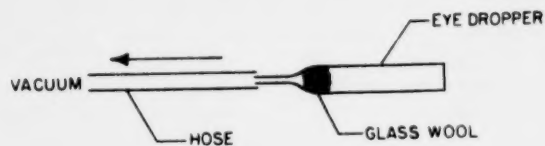


Figure 1. Diagram of Designation of Sections in the Cleanup and Separation of CCE-Aromatics on Silica Gel Layers.



SILICA GEL COLLECTION ASSEMBLY

FIGURE 2

REFERENCES

- 1 Smith, D. and Eichelberger, J. Thin-Layer Chromatography of Carbon Adsorption Extracts Prior to Gas Chromatographic Analysis for Pesticides. *J. Water Poll. Control Fed.* 37, 77, (1965).
- 2 Damaska, W. H. F.D.A. Cincinnati District, Private Communication. October, 1964.
- 3 Randerath, Kurt. *Thin-Layer Chromatography*, Academic Press. New York, 1963.
- 4 Bobbitt, J. M. *Thin-Layer Chromatography*. Theinhold, New York. 1963.

This outline was prepared by P. F. Hallbach, Chemist, National Training Center, Water Programs Operations, EPA, Cincinnati, OH 45268.

TABLE 1
SUGGESTED SYSTEMS FOR ADSORPTION CHROMATOGRAPHY

Compounds	Adsorbent	Developer	Visualization
1. Long-chain (over eight carbons) aliphatic ketones	silica gel G	a. benzene-EtOEt mixtures b. toluene-EtOEt mixtures c. pet. ether-EtOEt mixtures	phosphomolybdic acid (2, 4-DNPH)
2. Aliphatic and aromatic aldehydes and ketones	aluminum oxide	a. benzene b. benzene-EtOH (98:2) (95:5) (90:10) c. CHCl_3 d. EtOEt e. pet. ether-benzene (1:1)	2, 4-DNPH
3. Aromatic aldehydes and ketones	silica gel G	hexane-EtOAc (4:1) (3:2)	(U. V. on phosphors)
4. Vanillin and other substituted benzaldehydes	silica gel G	a. pet. ether-EtOAc (2:1) b. hexane-EtOAc (5:2) c. CHCl_3 -EtOAc (98:2) d. decalin- CH_2Cl_2 -MeOH (5:4:1)	(U. V. on phosphors)
5. 2, 4-Dinitrophenylhydrazones of aldehydes	silica gel G	a. benzene-pet. ether (3:1) for aliphatic b. benzene-EtOAc (95:5) for aromatic	colored
6. Miscellaneous alkaloids	silica gel G	a. CHCl_3 -acetone-diethylamine (5:4:1) b. CHCl_3 -diethylamine (9:1) c. cyclohexane- CHCl_3 -diethylamine (5:4:1) d. cyclohexane-diethylamine (9:1) e. benzene-EtOAc-diethylamine (7:2:1)	(Dragendorff's Reagent)
	aluminum oxide	f. CHCl_3 g. cyclohexane- CHCl_3 (3:7) plus 0.05% diethylamine	
7. Alkaloids and barbiturates in toxicology	silica gel G	a. MeOH b. CHCl_3 -EtOEt (85:15)	(Dragendorff's Reagent)
8. Strong-base amines	unbound aluminum oxide	a. acetone-heptane (1:1) b. $\text{CHCl}_3/\text{NH}_3$ (sat. at 22°)-EtOH (96%) (30:1)	I_2 vapor-U. V. (Dragendorff's Reagent)
9. Sugar acetates and inositol acetates	silica gel G	benzene with 2-10% MeOH	H_2O

TABLE 1 (continued)
SUGGESTED SYSTEMS FOR ADSORPTION CHROMATOGRAPHY

Compounds	Adsorbent	Developer	Visualization
10. Aldose 2, 4-DNPH's	a. aluminum oxide G b. silica gel G	a. toluene-EtOAc (1:1) b. toluene-EtOAc (3:1) (1:1)	a. colored compounds b. NaOH
11. Cardiac glycosides	silica gel G	a. CHCl_3 -MeOH (9:1) b. CHCl_3 -acetone (65:35)	H_2SO_4 and heat
12. Dicarboxylic acids	silica gel G	a. benzene-MeOH-HOAc (45:8:4) b. benzene-dioxane-HOAc (90:25:4)	bromphenol blue acidified with citric acid
13. p-Hydroxybenzoic acid esters	silica gel G	pentane-HOAc (88:12)	(U.V. on phosphors)
14. Sulfonamides	silica gel G	CHCl_3 -EtOH- heptane (1:1:1)	p-dimethylamino-benzaldehyde, acidified (U.V. on phosphors)
15. Food dyes	silica gel G	a. CHCl_3 -acetic anhydride (75:2) b. benzene c. methyl ethyl ketone-HOAc-MeOH (40:5:5)	SbCl_3 in CHCl_3
16. Misc. essential oils	silica gel G	benzene- CHCl_3 (1:1)	SbCl_3 in CHCl_3
17. Coumarins	silica gel G	a. pet. ether-EtOAc (2:1) b. hexane-EtOAc (5:2)	(U.V. on phosphors)
18. Alkali metals- Na^{1+} , Li^{1+} , K^{1+} , Mg^{2+}	purified silica gel G	EtOH-HOAc (100:1)	acid violet (1.5% aqueous soln.)
19. 24 ferrocene	silica gel G	a. benzene b. benzene-EtOH (30:1) (15:1) c. propylene glycol-MeOH (1:1) d. propylene glycol-chlorobenzene-MeOH (1:1:1)	(U.V. on phosphors)
20. Misc. lipids	silica gel G	a. EtOEt b. isopropyl ether c. isopropyl ether-HOAc (98.5:1.5)	(H_2SO_4 and heat)
21. Fatty acid methyl esters	silica gel G	hexane-EtOEt mixtures (up to 30% EtOEt)	a. I_2 b. H_2SO_4 and heat
22. Glycerides	silica gel G	a. Skellysolve F-EtOEt (70:30)	H_2SO_4 and heat

TABLE 1 (continued)
SUGGESTED SYSTEMS FOR ADSORPTION CHROMATOGRAPHY

Compounds	Adsorbent	Developer	Visualization
		b. (10:90) for monoglycerides c. (70:30) for diglycerides d. (90:10) for triglycerides e. (60:40) (35:65) (85:15)	
23. Long-chain aliphatic alcohols	silica gel G	a. pet. ether-EtOEt-HOAc (90:10:1) b. pet. ether-EtOEt (20:80) (10:90) (70:30)	H ₂ SO ₄ and heat
24. Phenols	a. starch-bound silicic acid kieselguhr (1:1)	a. xylene b. xylene-CHCl ₃ (3:1) (1:1) (1:3) c. CHCl ₃	(U.V. on phosphors)
25. Phenols and phenolic acids	starch-bound silicic acid with phosphors	a. Skellysolve B- EtOEt (3:7) b. Skellysolve B- EtOAc (1:3) c. Skellysolve B-acetone (3:1)	(U.V. on phosphors)
26. Phenols	plaster-of-Paris bound silica gel	a. hexane-EtOAc (4:1) (3:2) b. benzene-EtOEt (4:1) c. benzene	(U.V. on phosphors)
27. 3,5-Dinitrobenzoates of alcohols and phenols	silica gel G	a. benzene-pet. ether (1:1) b. hexane-EtOAc (85:15) (75:25) c. toluene-EtOAc (90:10)	colored compounds
28. Steroids	silica gel G	a. benzene b. benzene-EtOAc (9:1) (2:1) c. cyclohexane-EtOAc (9:1) (19:1) d. 1,2-dichloroethane	SbCl ₃ in CHCl ₃
29. 19-Nor-steroids	silica gel G	EtOAc-cyclohexane mixtures	SbCl ₃ in CHCl ₃
30. Blood cholesterol and cholesterol esters	silica gel G	a. benzene b. benzene-EtOAc (9:1) c. 1,2-dichloroethane d. CHCl ₃	SbCl ₃ in CHCl ₃
31. Triterpenoids	silica gel G	a. isopropyl ether-acetone (5:2) (19:1) b. isopropyl ether c. cyclohexane d. benzene e. CH ₂ Cl ₂	(H ₂ SO ₄ and heat)

TABLE 1 (continued)
SUGGESTED SYSTEMS FOR ADSORPTION CHROMATOGRAPHY

Compounds	Adsorbent	Developer	Visualization
32. Carotenes and fat-soluble vitamins, A, D, E, K	unbound aluminum oxide	a. MeOH b. CCl_4 c. xylene	H_2SO_4 and heat
33. Thiophene derivatives	a. silica gel G b. aluminum oxide G	a. benzene- CHCl_3 (9:1) b. MeOH c. pet. ether	(U. V. on phosphors)
34. Plasticizers (phthalates, phosphates and other esters)	silica gel G with phosphors	a. isooctane-EtOAc (90:10) b. benzene-EtOAc (95:5) c. butyl ether - hexane (80:20)	a. (U. V. on phosphors) b. I_2

INFRARED INSTRUMENTATION

I THEORY

A The Effect of Infrared Radiation on the Molecule

- 1 All molecules are made up of atoms connected by chemical bonds which act very much like springs.
- 2 Atoms or atomic groups are in continuous motion with respect to each other. Each molecule has specific vibrational frequencies for these motions.
- 3 A molecule which has the same vibrational frequency as the infrared radiation striking it will absorb that energy.
- 4 If the radiant frequency differs from the characteristic frequencies of the molecule, the radiation passes through the molecule undiminished.
- 5 The characteristic frequencies for particular molecules are determined by the masses of their atoms, their spatial geometry and the strength of their connecting bonds.

B Measuring Infrared Radiation

- 1 The infrared spectrum gives basic information about the molecular structure of a compound.
- 2 Organic chemicals are largely made up of different combinations of atomic building blocks called functional groups: -OH, -NH₂, -CH₃, -CO, -CN, -C-O-C-, -COOH.
- 3 These subgroups have characteristic absorption bands in certain parts of the infrared spectrum.
- 4 Spectra correlation charts provide a key to the location of characteristic absorption bands of the most common functional groups.

II THE INSTRUMENT

A Source

- 1 A length of ceramic tubing heated to about 1200°C
- 2 It produces a continuous spectrum of radiant energy, most of which falls within 2.5 to 15 micron region.

B Monochromator

- 1 It disperses the radiation into its component wavelengths.
- 2 Selects the particular wavelength interval transmitted to the detector.
- 3 Maintains, by varying slit widths, approximately constant energy through the slits to the detector.

C Wavelength

- 1 A mirrors rotational position determines the particular wavelength which emerges from the slit.
- 2 Linkage with the recorder drum shaft insures that the wavelength scale on the drum corresponds exactly to the wavelength selected.

D Resolution

- 1 A NaCl prism provides the best combination of dispersion and transmission in the operating range.
- 2 The sealed and desiccated monochromator protects the prism from moisture and dust.

E Thermocouple

- 1 A high sensitivity thermocouple consists of a blackened gold leaf target welded to two pins.

- 2 Temperature differences at the junctions of the leaf and pins set up a thermoelectric potential which is utilized.

F Optical Attenuator

- 1 By subtracting energy from the reference beam, the attenuator makes the amount of energy in the reference beam precisely the same as that in the sample beam.
- 2 This permits the pen to record directly in terms of sample transmittance.

G Instrument Performance

1 Scanning time

Twelve minutes for total scan, automatically programmed for constant rate per spectral slit width for constant accuracy over the wavelength range.

2 Accuracy

- a Abscissa $\pm 0.03\mu$, ordinate $\pm 1\%T$.
- b Less than 3% scattered light at 14.7μ and less than 1% from 2.5μ to 14μ .

III MICRO INFRARED TECHNIQUE

A Solution Technique

- 1 The technique most reliable for quantitative measurements in infrared region is the solution technique.
- 2 One drawback to this technique is the scarcity of suitable solvents with sufficient areas of transparency.

3 Carbon disulfide CS_2 is the most valuable of these because of its transparency in the "finger print" region of the infrared from $1,350$ to 650 cm^{-1} (7.4μ to 15μ). Carbon tetrachloride is a useful solvent from $4,000$ to $1,650\text{ cm}^{-1}$ (2.5μ to 6.0μ).

4 The sample is dissolved in 0.3 ml of solvent. The solution is then transferred to a 5 mm energy-path cavity cell by a hypodermic syringe. A matching cavity cell is used for solvent compensation.

5 With this technique, sensitivities down to $5\text{-}10\text{ }\mu\text{g}$ of strongly absorbing compounds are achieved without resorting to scale expansion.

B The Potassium Bromide KBr Pellet Technique

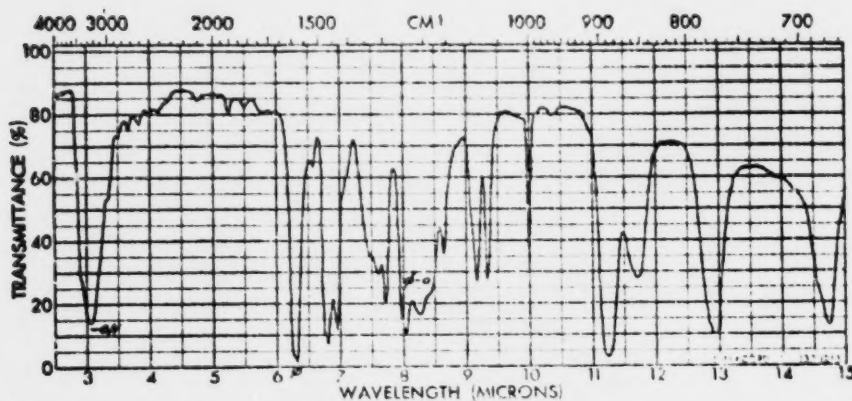
- 1 Since KBr is transparent throughout the commonly used portion of the infrared region, the entire infrared region is available as contrasted to the use of solvents.
- 2 A 6X beam-condenser is used to increase the energy transmitted through the pellet.
- 3 By this procedure a reliable sensitivity of about $5\mu\text{g}$ can easily be attained without scale expansion.

REFERENCES

- 1 Blinn, R. C. Infrared technique useful in residue chemistry, AOAC. Vol. 48, 1009-1017. 1965.
- 2 InfraCord, Perkin-Elmer spectrophotometer model 137-B Brochure, 1965.

This outline was prepared by J. W. Mandia, Chemist, formerly with National Training Center, MDS, WPO, EPA, Cincinnati, OH 45268.

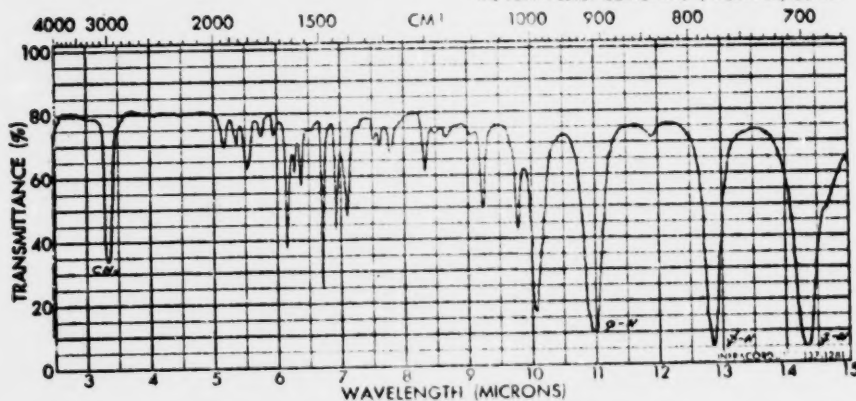
INFRARED IDENTIFICATION OF ORGANIC COMPOUNDS



SPECTRUM NO. <u>11</u>	ORIGIN	LEGEND	REMARKS
SAMPLE		1	<chem>CNc1ccccc1</chem>
<u>N-METHYLPHENOL</u>	PURITY <u>ACS</u>	2	
	PHASE <u>LIQUID</u>	DATE <u>3-20-59</u>	
	THICKNESS <u>5.0MM</u>	OPERATOR <u>DSB</u>	

SPECTRUM NO. 11

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.

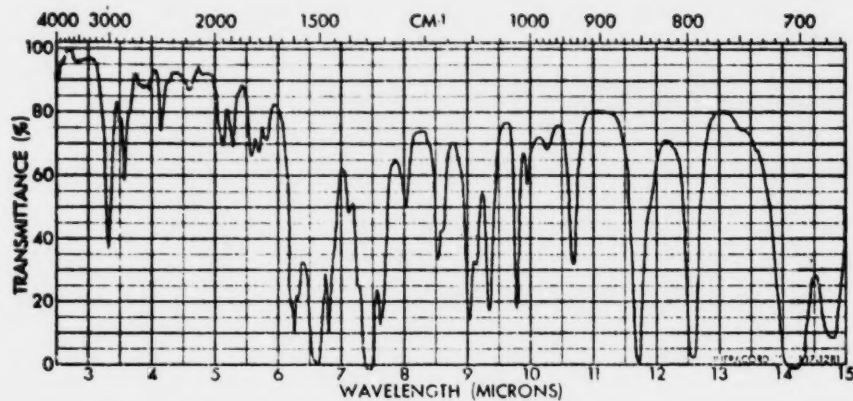


SPECTRUM NO. <u>12</u>	ORIGIN	LEGEND	REMARKS
SAMPLE		1	<chem>C=Cc1ccccc1</chem>
<u>STYRENE</u>	PURITY <u>??</u>	2	
	PHASE <u>LIQUID</u>	DATE <u>4-10-59</u>	
	THICKNESS <u>5.0MM</u>	OPERATOR <u>DSB</u>	

SPECTRUM NO. 12

CP, 075 (Rev. 1) - 6-6-55

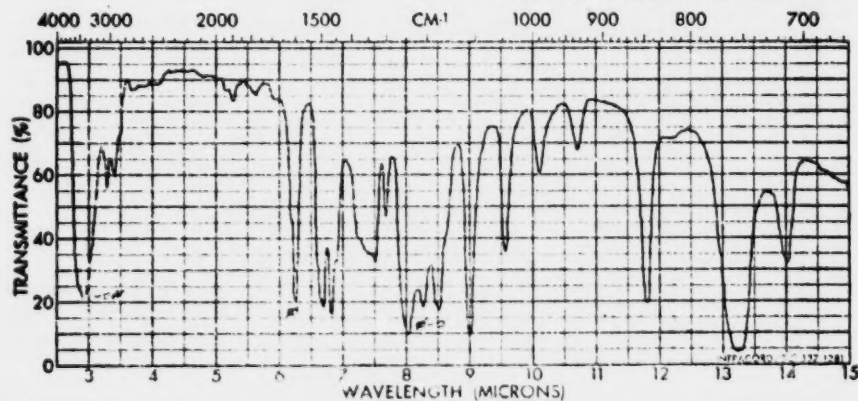
THE PERKIN-ELMER CORPORATION, NORWALK, CONN.



SPECTRUM NO. <u>9</u>	ORIGIN _____	LEGEND _____	REMARKS _____
SAMPLE _____	PURITY <u>ACS</u>	DATE <u>3-8-59</u>	
<u>NITROBENZENE</u>	PHASE <u>LIQUID</u>	OPERATOR <u>DGB</u>	
THICKNESS <u>0.25 MM</u>			

SPECTRUM NO. _____
SAMPLE _____

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.

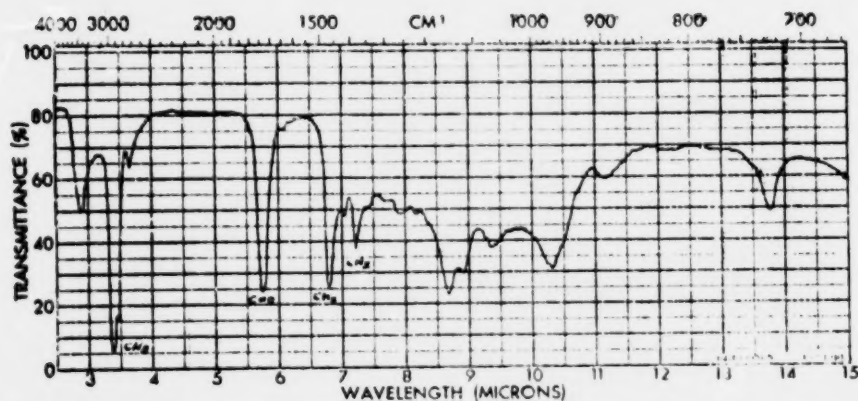


SPECTRUM NO. <u>22</u>	ORIGIN _____	LEGEND _____	REMARKS _____
SAMPLE _____	PURITY <u>ACS</u>	DATE <u>3-8-59</u>	
<u>0.25 MM</u>	PHASE <u>LIQUID</u>	OPERATOR <u>DGB</u>	
THICKNESS <u>0.25 MM</u>			

SPECTRUM NO. _____
SAMPLE _____

CHART 1400-1-9

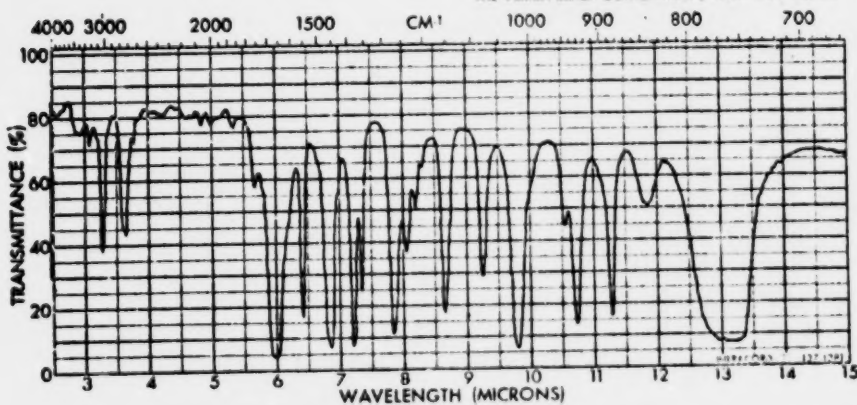
THE PERKIN-ELMER CORPORATION, NORWALK, CONN.



SPECTRUM NO. <u>7</u>	ORIGIN _____	LEGEND _____	REMARKS _____
SAMPLE _____	PURITY <u>??</u>	DATE <u>4-13-59</u>	CH ₃ (CH ₂) ₆ CHO
<u>OCTALDEHYDE</u>	PHASE <u>LIQUID</u>	OPERATOR <u>DGB</u>	
	THICKNESS <u>3.5MM</u>		

SPECTRUM NO. _____
SAMPLE _____

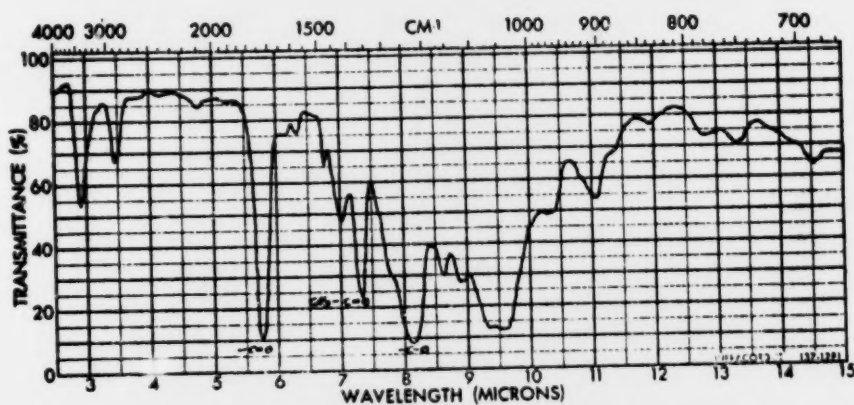
THE PERKIN-ELMER CORPORATION, NORWALK, CONN.



SPECTRUM NO. <u>8</u>	ORIGIN _____	LEGEND _____	REMARKS _____
SAMPLE _____	PURITY <u>??</u>	DATE <u>3/23/59</u>	CH ₂ =CH-C(=O)-CH=CH ₂
<u>FURFURAL</u>	PHASE <u>LIQUID</u>	OPERATOR <u>DGB</u>	
	THICKNESS <u>3.5MM</u>		

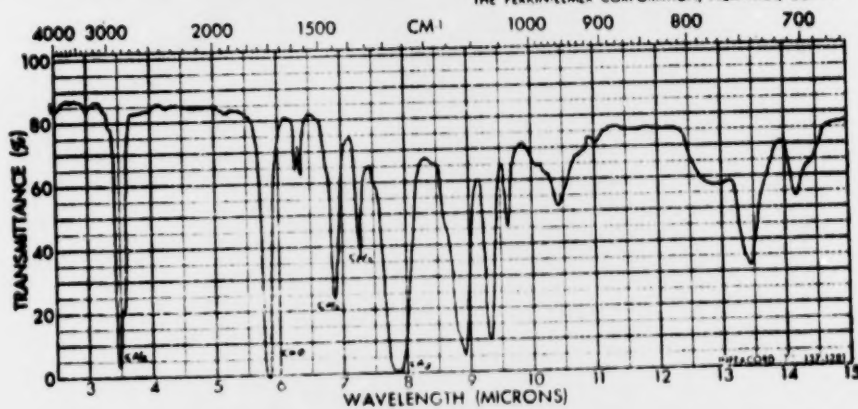
SPECTRUM NO. _____
SAMPLE _____CH₂(CH₂)₆CHO

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.



SPECTRUM NO. <u>5</u>	ORIGIN _____	LEGEND _____	REMARKS _____
SAMPLE _____	PURITY <u>??</u>	1. _____	$\text{CH}_3-\overset{\text{O}}{\parallel}{\text{C}}-\text{O}-$
<u>Acetate</u>	PHASE <u>gas</u>	2. _____	
_____	THICKNESS <u>shear</u>	DATE <u>4-1-59</u>	
_____	OPERATOR <u>DSB</u>		

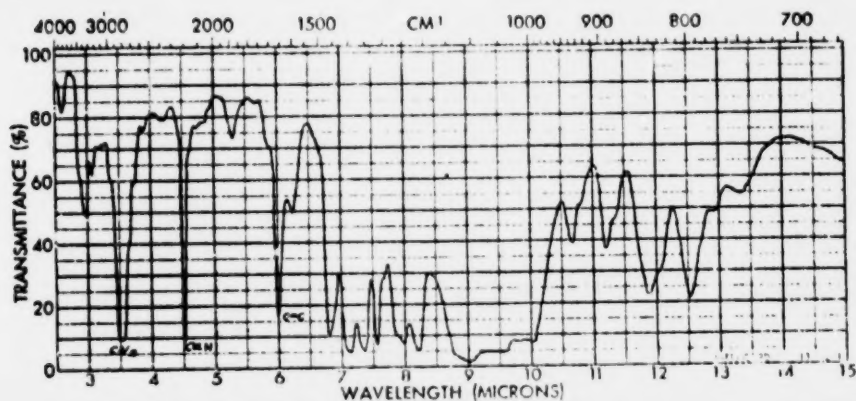
THE PERKIN-ELMER CORPORATION, NORWALK, CONN.



SPECTRUM NO. <u>6</u>	ORIGIN _____	LEGEND _____	REMARKS _____
SAMPLE _____	PURITY <u>??</u>	1. _____	<u>ISONITROBENZENE</u> $\text{C}_6\text{H}_5-\text{NO}_2$
<u>Isonitrobenzene</u>	PHASE <u>gas</u>	2. _____	
_____	THICKNESS <u>shear</u>	DATE <u>4-6-59</u>	
_____	OPERATOR <u>DSB</u>		

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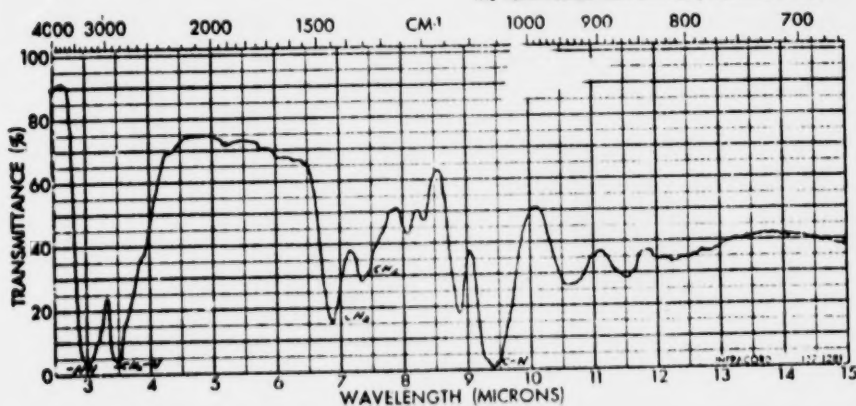
THE PERKIN-ELMER CORPORATION, NORWALK, CONN.



SPECTRUM NO. <u>3</u>	ORIGIN _____	LEGEND _____	REMARKS _____
SAMPLE _____	PURITY <u>ACS</u>	1 _____	<chem>NC(=O)Nc1ccc(C(=O)N)cc1</chem> OXYNICARBAMIDE
OXYNICARBAMIDE	PHASE <u>LIQUID</u>	2 _____	
_____	THICKNESS <u>0.2 mm</u>	DATE <u>4-3-59</u>	
_____	_____	OPERATOR <u>DSB</u>	_____

SPECTRUM NO. 3
SAMPLE _____

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.

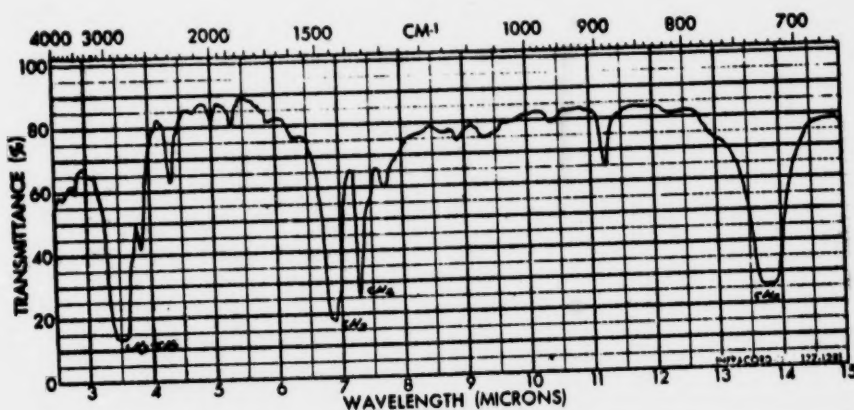


SPECTRUM NO. <u>47</u>	ORIGIN _____	LEGEND _____	REMARKS _____
SAMPLE <u>(4)</u>	PURITY <u>ACS</u>	1 _____	<chem>CCN(CC)CC</chem> DIETHYLAMINE
DIETHYLAMINE	PHASE <u>LIQUID</u>	2 _____	
_____	THICKNESS <u>5 mm</u>	DATE <u>1-2-59</u>	
_____	_____	OPERATOR <u>DSB</u>	_____

SPECTRUM NO. 47
SAMPLE _____

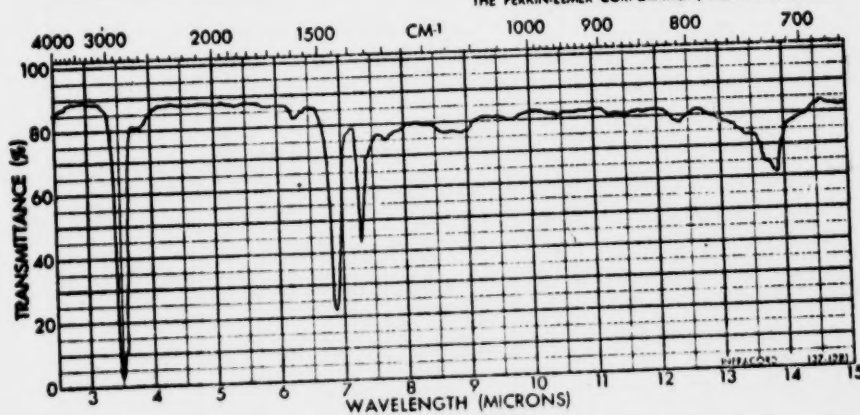
CH. 075.1ab.7.6.59

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.



SPECTRUM NO. <u>3</u>	ORIGIN _____	LEGEND _____	REMARKS _____
SAMPLE _____	PURITY <u>??</u>	1. _____	<u>-CH₂-CH₂-CH₂-</u>
<u>PARAFFIN WAXES</u>	PHASE <u>GAL</u>	2. _____	
_____	DATE <u>3-11-59</u>	_____	
_____	THICKNESS <u>SMEAR</u>	OPERATOR <u>DGB</u>	_____

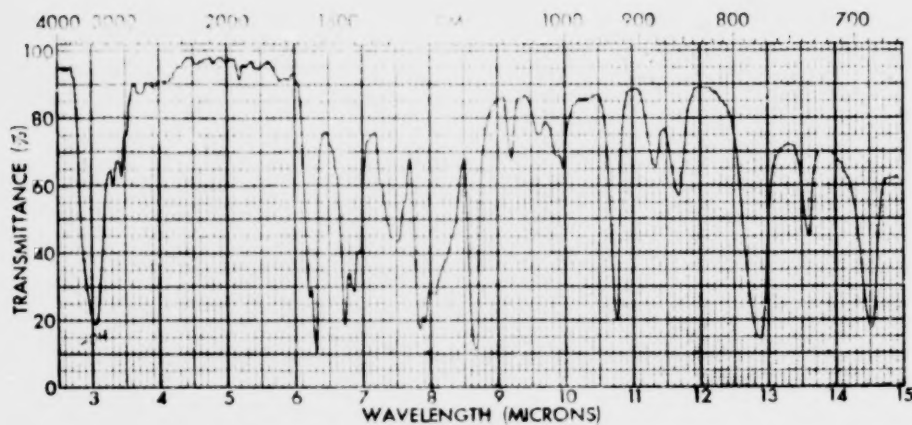
THE PERKIN-ELMER CORPORATION, NORWALK, CONN.



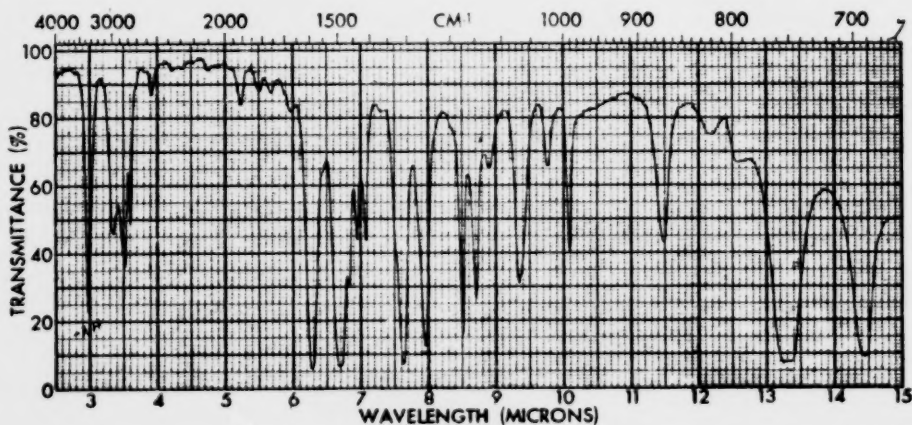
SPECTRUM NO. <u>2</u>	ORIGIN _____	LEGEND _____	REMARKS _____
SAMPLE _____	PURITY <u>—</u>	1. _____	_____
<u>PETROLEUM RESIDUE</u>	PHASE <u>GAL</u>	2. _____	
_____	DATE <u>2-11-59</u>	_____	
_____	THICKNESS <u>SMEAR</u>	OPERATOR <u>DGB</u>	_____

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.

CH. 075. 1ab. 1.6.59

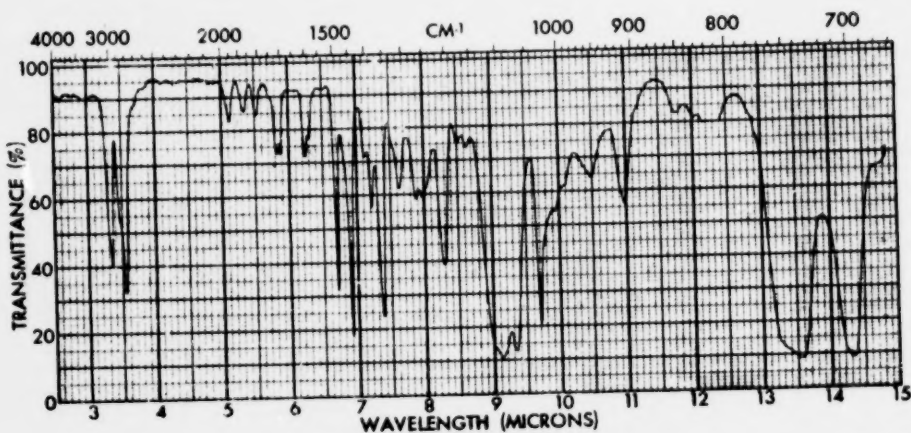


SAMPLE NO. <u>13</u>	REMARKS <u><chem>CH3</chem></u>	SAMPLE NO. _____ SOURCE _____ LOCATION _____
SOURCE <u>PURE LIQUID</u>		
LOCATION _____		
DATE SAMPLED _____	PHASE <u>LIQUID</u>	
TYPE <u>APPROX.</u>	OPERATOR <u>DAY</u>	
	DATE <u>3-14-63</u>	

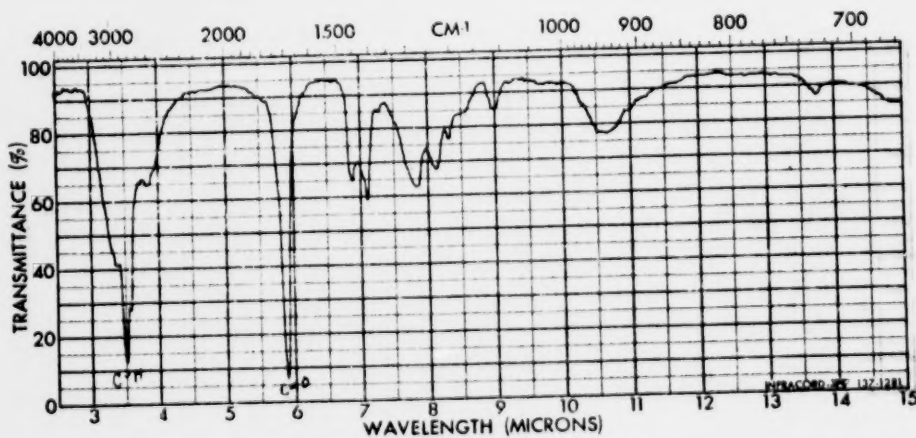


SAMPLE NO. <u>14</u>	REMARKS <u><chem>N(CH3)2</chem></u>	SAMPLE NO. _____ SOURCE _____ LOCATION _____
SOURCE <u>N-METHYL ANILINE</u>		
LOCATION _____		
DATE SAMPLED _____	PHASE <u>LIQUID</u>	
TYPE <u>SANDWICH</u>	OPERATOR <u>DAY</u>	
	DATE <u>3-14-63</u>	

Infrared Identification



SAMPLE NO. <u>15</u>	REMARKS <u><chem>c1ccccc1COCC</chem></u>	SAMPLE NO. _____ SOURCE _____ LOCATION _____
SOURCE <u>PURE BENZYL ETHER</u>		
LOCATION _____		
DATE SAMPLED _____	PHASE <u>LIQUID</u>	
TYPE <u>SANDWICH</u>	OPERATOR <u>GAP</u>	
	DATE <u>3-10-63</u>	



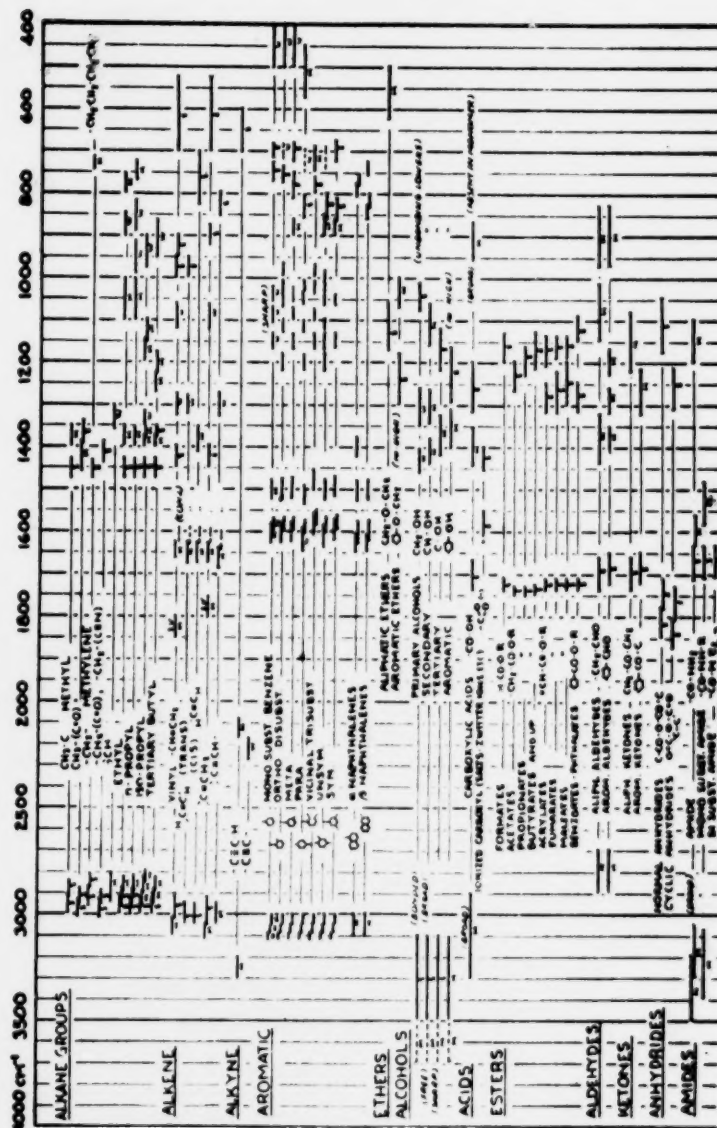
SPECTRUM NO. <u>16</u>	ORIGIN _____	LEGEND _____	REMARKS <u><chem>CH3(CH2)9COOH</chem></u>	SPECTRUM NO. _____ SAMPLE _____
SAMPLE <u>10-UNDENOIC ACID</u>		1. _____		
	PURITY <u>PURE</u>	2. _____		
	PHASE <u>LIQUID</u>	DATE <u>2-11-63</u>		
	THICKNESS <u>0.118</u>	OPERATOR <u>GAP</u>		

THE PERKIN-ELMER CORPORATION, NORWALK, CONN.

DATA SHEET - ORGANIC LABORATORY

SOLUBILITY SEPARATION		CHROMATOGRAPHIC SEPARATION OF NEUTRALS	
WEIGHTS	REMARKS	WEIGHTS	REMARKS
ORIGINAL SAMPLE _____ GM		ALIQOT USED _____ _____ GM	
BASES _____ _____ GM		ALIPHATICS _____ _____ GM	
STRONG ACIDS _____ _____ GM		AROMATICS _____ _____ GM	
WEAK ACIDS _____ _____ GM		OXYS _____ _____ GM	
NEUTRALS _____ _____ GM		SUMMARY OF DATA BASES _____ GM STRONG ACIDS _____ WEAK ACIDS _____ NEUTRALS _____ TOTAL RECOVERED _____ GM % RECOVERY _____ %	

N. B. COLTHUP



SPECTRA-STRUCTURE CORRELATIONS

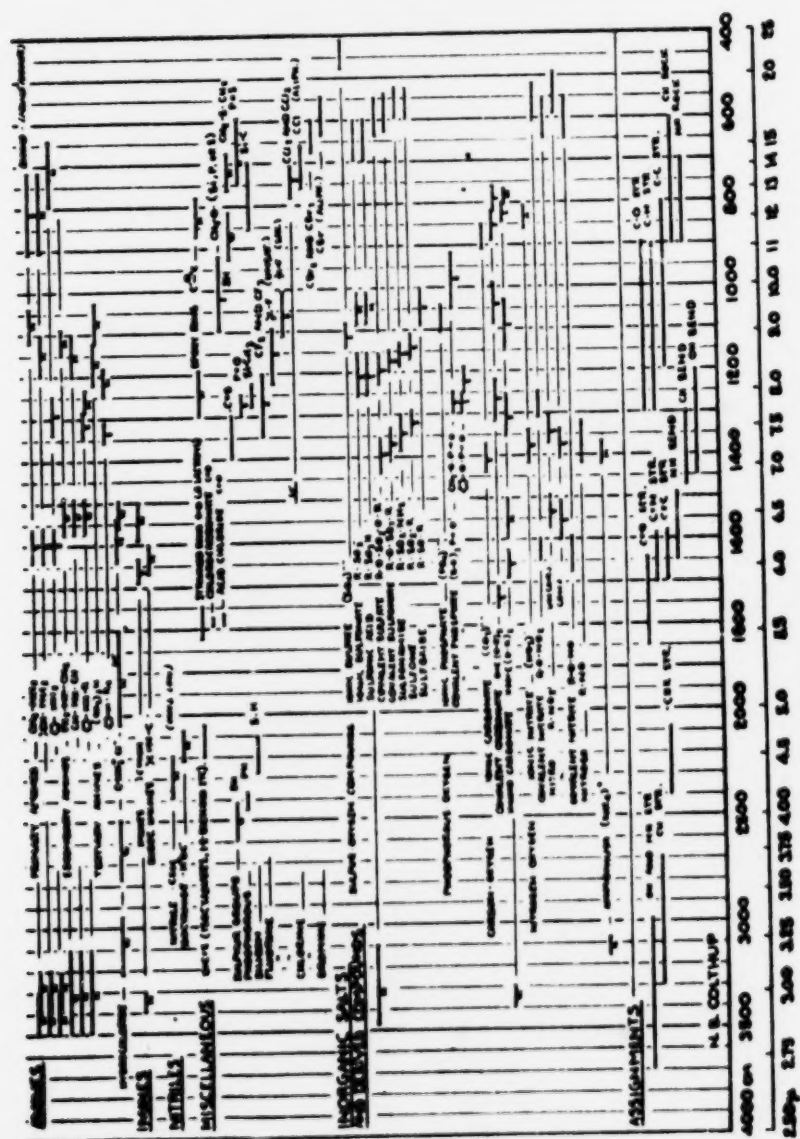


Chart 1. Probable positions of characteristic infra-red absorption bands.

Chart 1. From Colthup, N.B. "Spectra-Structure Correlations in the Infra-Red Region".
Journal of the Optical Society of America. 40:397-400. June, 1950.

VIII. SPECIAL TOPICS

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WATER QUALITY SURVEYS

I INTRODUCTION

A Theoretical Approach

- 1 This discussion will present as a base, the procedures for the conduct of an ideal water quality survey.

B Practical Limitations

- 1 In practice, limitations of personnel, facilities, time and money, always will require compromise between the ideal and the possible.

C Objectives of Quality Studies

- 1 The four basic objectives behind the almost limitless number of possible reasons for water quality studies are:
 - a Determination of the natural water quality of the stream.
 - b Measurement, in a selected and limited period of time, of the existing effects of wastes on water quality and uses.
 - c Procurement of data on waste loads, water quality and stream characteristics that will permit projection of the data to describe probable water quality and effects on uses under a variety of conditions other than those that prevailed during the study.
 - d Determination of corrective measures needed to protect the stream water quality for proper uses.

II PRELIMINARY PLANNING

A Available Information

Assemble and review all readily available maps, information, and reports bearing on the stream under consideration.

B Problem and Objectives of Study

- 1 Define the problem requiring study as completely as possible on the basis of available information.
- 2 Prepare a list covering all known objectives that may be achieved by the study.

C Tentative Plan of Study

- 1 Prepare a brief preliminary plan of study for guidance in the subsequent field reconnaissance.
- 2 Include in the preliminary plan:
 - a Locations and strengths of known sources of waste
 - b Locations of areas of water use and a list of legitimate water uses
 - c Section of major stream and locations of important tributaries and unique features such as dams and points of major diversions
 - d Possible sampling stations
 - e Sampling frequency and number of samples at each station

- f Types of laboratory determinations
- g Existing stream gauging stations and additional points where stream flow data are needed.
- h Other hydrological data needed and possible means of procurement
- i Potential laboratory locations
- j Special supplies and equipment
- k Personnel requirements
- l Approximate cost of field operations

III PRELIMINARY FIELD OPERATIONS

A Importance

This step is often neglected, but a small advance party of two or three experienced field men can obtain information and make preparations that will save time and money and avoid much confusion and possible error when the subsequent field study starts.

B Local Contacts

Contact local agencies and individuals who use or have knowledge of the stream, and assemble available maps, reports, operating records, and verbal information.

C Sources of Wastes

1 Sewage

- a Obtain best available estimate of sewered population.
- b Examine sewage treatment plant, if any, and obtain copies of operating records on volumes and characteristics of sewage.
- c Locate all points of discharge and make any installations necessary for sampling and gauging.

2 Industrial wastes

- a Investigate and prepare flow diagram of process with especial reference to points of water use and waste discharge, and quantities of raw materials and finished products.
- b Obtain information on plant operating schedule, with especial attention to daily or seasonal variations, and to any anticipated changes in process or increase in production.
- c Obtain data on water consumption, numbers of plant employees by work shifts, and disposal of domestic sewage.
- d Investigate waste treatment facilities, if any, and obtain operating records on volumes and characteristics of wastes.
- e Locate all points of waste discharge, trace origins in plant process, and make any installations necessary for sampling and gauging.
- f Collect samples for preliminary examination in laboratory to permit selection of proper analytical procedures.

D Stream

- 1 Observe entire reach of stream involved by wading, walking bank, or boat.
- 2 Note especially points of waste discharge and dispersion patterns, visual evidences of pollution confluences and mixing of tributaries, stream flow characteristics, approximate widths and depths, and locations of prospective sampling stations, and of water uses.
- 3 Collect bottom samples of biological organisms and bottom deposits, and observe evidences of algae.

- a Bottom organisms serve as an index of degree of pollution, and of length of river that should be sampled.
 - b Bottom deposits indicate extent of sludge deposits.
 - c Algae indicates probable significance of photosynthesis and possible need for night sampling or light-dark bottle technique for full evaluation of dissolved oxygen variations.
- 4 Determine times of water travel between pertinent points on stream.
- a Knowledge of times of water travel not only will permit the most intelligent conduct of study, but also is essential to the complete analysis, interpretation, and projection of data.
 - b Time and effort involved are a minor portion of the totals devoted to the stream study, and once established, need not be repeated in subsequent studies.
 - c Observations should be made at three or more stream flows to develop a time of travel versus stream discharge curve, which will permit interpolation for any desired stream flow.
 - d Methods include: tracing an inherent or added variable stream constituent; timing surface or submerged floats; and determining stream cross sections at selected intervals.
- 5 Select and identify sampling stations
- a For DO evaluation on the majority of streams, there should be: one station just above each major waste source; stations at about half day intervals for two days time of travel below waste sources; and stations at about one day intervals for an additional three to four days time of travel.
 - b For coliform evaluation, the stations should be similar to those for DO except that the downstream stations at one day intervals should extend to a total of 10 to 20 days time of water travel below the source of wastes.
 - c In very small or very turbulent streams, natural purification may be well advanced in as little as one to two days time of water travel and stations should be at much closer intervals than suggested above.
 - d If tributary streams are important, there should be one station near the mouth of each tributary and one on the main stream just above each confluence.
 - e Stations should be established as close as practicable to points or areas of important water uses.
 - f At stations where wastes and tributary waters are well mixed, one sampling point near mid-channel usually is adequate.
 - g At stations where mixing is inadequate, common practice is to sample at the quarter points of the stream.
 - h Identify stations by adequate description and marking, and make any necessary preparations for use by sampler.
 - i Select best access routes to sampling stations and make a round-trip to stations to determine time required for sampling.
- E Preliminary Stream Samples
- 1 Collect one or more sets of stream samples for preliminary examination.
 - a Dissolved oxygen results will be useful in establishing sampling stations and length of stream that should be studied

b Determinations of coliform organisms and BOD will assist in selection of proper dilutions for subsequent use when study is started.

c Determinations of other constituents will enable analyst to select most acceptable laboratory procedures and to determine sizes of sample portions necessary for those analyses in which concentrations of constituents govern choice of portion sizes.

F Laboratory Location

Examine potential laboratory locations in relation to convenience to the field operations, adequacy of space and utilities, storage space, and revisions or additional facilities that will be needed.

G Miscellaneous

- 1 Locate local sources of supplies that will be purchased during study.
- 2 Determine transportation routes and schedules for shipments of samples, supplies, and materials.
- 3 Arrange for local personnel and special facilities that will be used.
- 4 Investigate rooming facilities convenient to the field operations.

IV FINAL PLANNING

A Revision of Preliminary Plan

With the preliminary plan as a guide, and with the knowledge gained from the preliminary field operations it will be possible to prepare an intelligent, workable plan for the field study.

B Objectives of Study

Redefine, add to, or delete from, the initial list of objectives, and prepare a set of specific objectives which will include provisions for all essential

answers to the problem at hand, but will also eliminate needless expenditure, on nonessential matters, of effort which might better be devoted to the principal problem.

C Period of Field Operations

- 1 The overall period selected for the study should be at the time of year when past experience indicates that streamflows usually are relatively stable.
- 2 For evaluation of DO depletion; a period of warm weather and low streamflow is desirable.
- 3 For evaluation of coliform contamination, a period of intermediate flow may be desirable.
- 4 A period of intensive round-the-clock sampling for a few days generally is preferable to a period of a month of sampling daily or on alternate days, but a combination of both methods may be better than either one.

D Sampling

1 General

- a If sources of wastes do not vary significantly during period of study, the wastes may be sampled in advance of the stream study.
- b Sampling procedures and preservation of samples should follow "Standard Methods for the Examination of Water and Wastewater" and/or procedures recommended by the investigators agency.

2 Sewage

- a Collect samples around-the-clock at 15 minute to 1 hour intervals for three to seven days.
- b Composite sample portions in portion to flow for three to four equal time periods of each day.

3 Industrial wastes

- a In general, the better known the process is, and the less variable the waste discharge, the less detailed need the sampling be.
- b For well known processes with little variation, equal sample portions collected at one-half to one hour intervals and composited for eight hours or for a complete cycle of operation on three to five days should be adequate.
- c For little known and variable processes, detailed and prolonged sampling may be necessary, with samples collected at 5 to 15 minute intervals, and composited in proportion to flow for six to 24 hour periods for seven or more days or complete cycles of operation.

4 Stream

- a If streamflow, waste discharge, or oxygen production by algae vary widely throughout the day, sample around-the-clock at several stations for at least one or two days to establish the daily cycle of variation in waste constituents and effects.
- b In most streams, grab samples each day or on alternate days for two weeks to a month is adequate if conditions remain stable.
- c The total number of samples collected from a single station during a field study generally ranges between 15 and 20. In the absence of wide variations in streamflow, 12 to 15 sets of samples usually yield adequate, usable results.
- d If sampling is not around the clock, sample collection should be varied throughout the day as much as is feasible, and as a minimum, the direction of the sampling trip should be reversed on alternate days.
- e When more than one point is sampled at a station, individual field deter-

minations are made and a separate bacteriological sample is taken at each point, but samples for other determinations may be combined in a single composite. Bacteriological samples from two or more points at the same station may be composited in the laboratory.

E Gauging

- 1 Methods of waste and stream gauging are covered in another reference outline.
- 2 Generally stream discharge records are obtained from existing U. S. Geological Survey stations, or from special stations established and rated by them upon request.
- 3 Streamflow data at each main stream sampling station generally can be computed with sufficient accuracy from records for one or two main stream gauging stations and one for each major tributary.

F Laboratory Operations

- 1 Select principal determinations that will measure pertinent waste constituents and their effects, and auxiliary determinations that will contribute to interpretation of principal results.
- 2 Reject determinations that would be "interesting" but would not contribute to solution of the problem.
- 3 Generally useful principal determinations common to many stream studies are those for coliform bacteria, bottom organisms, temperature, DO, and BOD, and auxiliary determinations frequently are for pH, alkalinity, and turbidity.

Other determinations must be selected for special purposes and for special types of wastes.
- 5 Laboratory methods should follow procedures recommended in "Standard Methods for the Examination of Water and Wastewater."
- 6 Avoid excessive overload of the laboratory by working closely with the

laboratory supervisor during the planning stage and accepting his estimate of the volume of work that can be handled.

- 7 In establishing the laboratory work load, allow time for calculation of analytical results at the end of each day.

- 8 Ship samples to the headquarters laboratory for all determinations of stable or preserved constituents that do not have to be made in the field.

G Personnel

- 1 The field crew should possess competencies for the specific tasks involved, or should be trained in advance if necessary.

- 2 Personnel needed commonly will include:

a Sanitary engineers

b Chemists

c Bacteriologists

d Biologists

e Sampling and laboratory aides

H Supplies and Equipment

- 1 Prepare and use a check list of needed field supplies and equipment.
- 2 All operations that can be performed in advance at headquarters, such as training, purchases, equipment repair, and reagent preparation will save money and valuable time in the field.

I Cost Estimate

- 1 Revise the initial estimate of cost of field operations in light of the final plan adopted.

- 2 Adjust the final plan to fit the money available for the study, or arrange for the additional money needed.

V FIELD OPERATIONS

A Briefing

- 1 Assemble the field crew and explain the problem, objectives, and details of field operations.
- 2 Take at least key personnel on a tour of pertinent physical features of the study.
- 3 Specify responsibilities of individuals for the various phases of the operation.

B Communications

Arrange a system of communication by which any member of the crew can be contacted within a reasonable length of time.

C Records

- 1 A few simple field and laboratory forms will serve to systematize the maintenance of basic records.
- 2 Keep all permanent field and laboratory records in bound volumes.
- 3 Encourage the recording of other than routine observations by both sampling and laboratory personnel.
- 4 Write down at once, and do not depend on memory for all significant observations.

D Revision of Operations

- 1 Review all daily data accumulated at the end of each day, and note especially any omissions or unusual results.

- 2 On the basis of the daily review, consider the need to:
 - a Add new determinations if needed.
 - b Omit determinations that are not showing significant results.
 - c Revise analytical methods.
 - d Change sampling stations or scheduled times of sampling.
 - e Investigate causes of apparently abnormal or erroneous data.
- 3 Do not revise operations unless the data or other new information indicates changes are essential to achieve the desired objectives.

VI USE OF DATA

A Analysis and Interpretation

- 1 The raw data are of only limited value until submitted to analysis and interpretation by an experienced person who is familiar with the problem involved.
- 2 Methods of analysis and interpretation constitute a major subject in themselves and are covered by other lectures.

- 3 No amount of statistical manipulation of the data can produce sound conclusions unless the stream study is soundly conceived, and carefully and conscientiously executed.

B Report

A report should be prepared if the data are to achieve their maximum usefulness, since raw data in dead files only rarely benefit anyone.

C Ultimate Disposal

After use, the data should be filed, indexed, and described so that they can be available and understandable years later to others not directly associated with the study.

D Maximum Usefulness

The results of a stream pollution study yield their maximum benefit and return their greatest satisfaction to those who worked to obtain them only when the results serve as a basis for actual correction of an abuse of stream water quality.

REFERENCE

Kittrell, F.W. A Practical Guide to Water Quality Studies of Streams. USDI, FWPCA, CWR-5. 1969.

This outline was prepared by Francis W. Kittrell, former Special Consultant, National Field Investigations Center, --Cincinnati Office of Enforcement and General Counsel, EPA, Cincinnati, OH 45268.

SAMPLE HANDLING - FIELD THROUGH LABORATORY

I PLANNING A SAMPLING PROGRAM

A Factors to Consider:

- 1 Locating sampling sites
- 2 Sampling equipment
- 3 Type of sample required
 - a grab
 - b composite
- 4 Amount of sample required
- 5 Frequency of collection
- 6 Preservation measures, if any

B Decisive Criteria

- 1 Nature of the sample source
- 2 Stability of constituent(s) to be measured
- 3 Ultimate use of data

II REPRESENTATIVE SAMPLES

If a sample is to provide meaningful and valid data about the parent population, it must be representative of the conditions existing in that parent source at the sampling location.

- A The container should be rinsed two or three times with the water to be collected.

B Compositing Samples

- 1 For some sources, a composite of samples is made which will represent the average situation for stable constituents.
- 2 The nature of the constituent to be determined may require a series of separate samples.

- C The equipment used to collect the sample is an important factor to consider. ASTM⁽¹⁾ has a detailed section on various sampling devices and techniques.

- D Great care must be exercised when collecting samples in sludge or mud areas and near benthic deposits. No definite procedure can be given, but careful effort should be made to obtain a representative sample.

III SAMPLE IDENTIFICATION

- A Each sample must be unmistakably identified, preferably with a tag or label. The required information should be planned in advance.

- B An information form preprinted on the tags or labels provides uniformity of sample records, assists the sampler, and helps ensure that vital information will not be omitted.

C Useful Identification Information includes:

- 1 sample identity code
- 2 signature of sampler
- 3 signature of witness
- 4 description of sampling location detailed enough to accommodate reproducible sampling. (It may be more convenient to record the details in the field record book).
- 5 sampling equipment used
- 6 date of collection
- 7 time of collection
- 8 type of sample (grab or composite)
- 9 water temperature
- 10 sampling conditions such as weather, water level, flow rate of source, etc.
- 11 any preservative additions or techniques
- 12 record of any determinations done in the field
- 13 type of analyses to be done in laboratory

IV SAMPLE CONTAINERS

A Available Materials

- 1 glass
- 2 plastic
- 3 hard rubber

B Considerations

- 1 Nature of the sample - Organics attack polyethylene.
- 2 Nature of constituent(s) to be determined
- Cations can adsorb readily on some plastics and on certain glassware. Metal or aluminum foil cap liners can interfere with metal analyses.
- 3 Preservatives to be used - Mineral acids attack some plastics.
- 4 Mailing Requirements - Containers should be large enough to allow extra volume for effects of temperature changes during transit. All caps should be securely in place. Glass containers must be protected against breakage. Styrofoam linings are useful for protecting glassware.

C Preliminary Check

Any question of possible interferences related to the sample container should be resolved before the study begins. A preliminary check should be made using corresponding sample materials, containers, preservatives and analysis.

D Cleaning

If new containers are to be used, preliminary cleaning is usually not necessary.

If the sample containers have been used previously, they should be carefully cleaned before use.

There are several cleaning methods available. Choosing the best method involves careful consideration of the nature of the sample and of the constituent(s) to be determined.

- 1 Phosphate detergents should not be used to clean containers for phosphorus samples.

- 2 Traces of dichromate cleaning solution will interfere with metal analyses.

E Storage

Sample containers should be stored and transported in a manner to assure their readiness for use.

V SAMPLE PRESERVATION

Every effort should be made to achieve the shortest possible interval between sample collection and analyses. If there must be a delay and it is long enough to produce significant changes in the sample, preservation measures are required.

At best, however, preservation efforts can only retard changes that inevitably continue after the sample is removed from the parent population.

A Functions

Methods of preservation are relatively limited. The primary functions of those employed are:

- 1 to retard biological action
- 2 to retard precipitation or the hydrolysis of chemical compounds and complexes
- 3 to reduce volatility of constituents

B General Methods

- 1 pH control - This affects precipitation of metals, salt formation and can inhibit bacterial action.
- 2 Chemical Addition - The choice of chemical depends on the change to be controlled.

Mercuric chloride is commonly used as a bacterial inhibitor. Disposal of the mercury-containing samples is a problem and efforts to find a substitute for this toxicant are underway.

To dispose of solutions of inorganic mercury salts, a recommended procedure is to capture and retain the mercury salts as the sulfide at a high pH. Several firms have tentatively agreed to accept the mercury sulfide for re-processing after preliminary conditions are met.⁽⁴⁾

- 3 Refrigeration and Freezing - This is the best preservation technique available, but it is not applicable to all types of samples. It is not always a practical technique for field operations.

C Specific Methods

The EPA Methods Manual⁽²⁾ includes a table summarizing the holding times and preservation techniques for several analytical procedures. This information also can be found in the standard references^(1, 2, 3) as part of the discussion of the determination of interest.

VI METHODS OF ANALYSIS

Standard reference books of analytical procedures to determine the physical and chemical characteristics of various types of water samples are available.

A EPA Methods Manual

The Analytical Quality Control Laboratory, National Environmental Research Center - Cincinnati, Environmental Protection Agency, has published a manual of analytical procedures to be used in Water Programs Operations laboratories for the analysis of water and wastes. The title of this manual is "Methods for Chemical Analysis of Water and Wastes" (1971).

For some procedures, the analyst is referred to Standard Methods and/or to ASTM Standards.

B Standard Methods

The American Public Health Association, the American Water Works Association and the Water Pollution Control Federation

prepare and publish a volume describing methods of water analysis. These include physical and chemical procedures. The title of this book is "Standard Methods for the Examination of Water and Wastewater", 13th edition, 1971.

C ASTM Standards

The American Society for Testing and Materials publishes an annual "book" of specifications and methods for testing materials. The "book" currently consists of 33 parts. The part applicable to water is a book titled, "Annual Book of ASTM Standards, Part 23, Water; Atmospheric Analysis".

D Other References

Current literature and other books of analytical procedures with related information are available to the analyst.

VII ORDER OF ANALYSES

The ideal situation is to perform all analyses shortly after sample collection. In the practical order, this is rarely possible. The allowable holding time for preserved samples is the basis for scheduling analyses.

- A The allowable holding time for samples depends on the nature of the sample, the stability of the constituent(s) to be determined and the conditions of storage.

- 1 For some constituents and physical values, immediate determination is required, e.g. dissolved oxygen, pH.

- 2 Using preservation techniques, the holding times for other determinations range from 6 hours (BOD) to 7 days (COD). Metals may be held up to 6 months.⁽²⁾

- 3 The EPA Methods Manual⁽²⁾ includes a table summarizing holding times and preservation techniques for several analytical procedures. This information can also be found in the standard

references^(1, 2, 3) as part of the discussion of the determination of interest.

- 4 If dissolved concentrations are sought, filtration should be done in the field if at all possible. Otherwise, the sample is filtered as soon as it is received in the laboratory. A 0.45 micron membrane filter is recommended for reproducible filtration.

- B The time interval between collection and analysis is important and should be recorded in the laboratory record book.

VIII RECORD KEEPING

The importance of maintaining a bound, legible record of pertinent information on samples cannot be over-emphasized.

A Field Operations

A bound notebook should be used. Sample information written in it would include:

- 1 Sample identification records (See Part III)
- 2 Any information requested by the analyst as significant
- 3 Details of sample preservation
- 4 A complete record of data on any determinations done in the field. (See B, next)
- 5 Shipping details and records

B Laboratory Operations

Samples should be logged in as soon as received and the analyses performed as soon as possible.

A bound notebook should be used. Preprinted data forms provide uniformity of records and help ensure that required information will be recorded. Such sheets should be permanently bound.

Items in the laboratory notebook would include:

- 1 sample identifying code
- 2 date and time of collection
- 3 date and time of analysis
- 4 the analytical method used
- 5 any deviations from the analytical method used
- 6 data obtained during analysis
- 7 quality control checks used
- 8 any information useful to those who interpret and use the data
- 9 signature of the analyst

IX SUMMARY

Valid data can be obtained only from a representative sample, unmistakably identified, carefully collected and stored. A skilled analyst, using reliable methods of analyses and performing the determinations within the prescribed time limits, can produce data for the sample. This data will be of value only if a written record exists to verify sample history from the field through the laboratory.

REFERENCES

- 1 ASTM Standards, Part 23, Water; Atmospheric Analysis.
- 2 Methods for Chemical Analysis of Water and Wastes, EPA-AQCL, 1971, Cincinnati, OH 45268.
- 3 Standard Methods for the Examination of Water and Wastewater, 13th edition, APHA-AWWA-WPCF, 1971.
- 4 Dean, R., Williams, R. and Wise, R., Disposal of Mercury Wastes from Water Laboratories, Environmental Science and Technology, October, 1971.

This outline was prepared by A. Donahue, Chemist, National Training Center, DTTB, MDS, WPO, EPA, Cincinnati, OH 45268.

AUTOMATION OF CHEMICAL ANALYSIS

I INTRODUCTION

Environmental pollution control and monitoring have become, in very recent years, the focal point of national and international attention. The analytical chemist has an important role in matters regarding pollution, for he determines not only the amount of pollution in air or water but also the efficiency of prevention and treatment methods. The increasing number of samples and of measurements to be made requires him to employ every means possible to keep abreast with the demands.

The need for instrumental and automated analytical techniques is well recognized. Automated sensors with continuous readout for the measurement of key constituents associated with pollution would be ideal.

II DEGREES OF AUTOMATION

- A Maximum automation - continuous sampling direct from source, with telemetering of results to central computer.
- B Medium automation - grab samples in laboratory with multiple simultaneous instrumental analysis, recording, calculating and digital print-out of data.
- C Minimum automation - maximum simplicity and efficiency of manual analytical methods.

Possible considerations are:

- 1 Automatic pipettes, burettes and reagent dispensers
- 2 Choice of glassware - precision and accuracy of graduated test tubes and cylinders vs. volumetric flasks should be compared to the accuracy of the analytical method.
- 3 Electrical "on and off" timers on hot plates (C. O. D.) and automated instruments (Technicon).

4 Use of semi-micro methods:

- a TKN - digest and distill 50 ml sample in place of 500 ml.
- b B.O.D. - incubate 125 ml samples in place of 300 ml.

5 Combining reagents:

- a Nitrite - reagent contains buffer, diazotization and coupling reagent.
- b Phosphorus - reagent contains acid, molybdate and reductant.

6 Laboratory management - elimination of needless repetitious work

- a Lab's sample log number vs. engineer's coding system
- b Distributing samples to instrumental stations in permanent labeled containers
- c Permanent data sheets vs. individual slips of paper for record keeping.
- d Washing glassware - cleaning acid vs. soap; labor costs
- e Documented laboratory procedures, techniques, and practices to avoid variations.

III SENSORS AND INSTRUMENTATION

In order to measure the amount of any constituent in a liquid or gaseous medium on an automated system, a relationship between the parameter and an electrical signal must be established. When such a relationship is established and is not affected by uncontrollable variables, electronic technology already available can be applied to the sensor's signal for digital print-out of concentration or for telemetering and conversion of the signal to meaningful data through computerization.

Various types of sensors and instrumental methods for water quality parameters are shown in Table 1. Parameters whose measurement is based on an electrochemical principle are very desirable because of the possibility of developing an electrode or probe. In such a case, no intermediate chemical reaction is needed for the ion in the sample to produce a signal.

- A Probes for many specific ions are available; however, most are affected by interferences and variables. The activity of the specific ion, sensed by the electrodes, is related to its concentration by the activity coefficient, which depends on the total ionic strength of the solution⁽⁴⁾. By increasing the ionic strength in all the samples, the effect of individual variation between samples can be eliminated.

With some specific ion probes, a pH adjustor and a complexing agent must also be added to the samples to eliminate interference from hydrogen, hydroxyl and metal ions.

Specific ion probes are valuable in some applications but must be evaluated for such use. Most of the automated continuous monitoring systems for water quality that are now available use sensors which require no intermediate chemical reaction and only measure parameters such as temperature, pH, conductivity, solar radiation, turbidity, chlorides and dissolved oxygen.

- B Automatic Titrators with preset end point are applicable for many measurements using various types of electrodes. Up to 10 samples can be titrated without attendance.
- C Atomic Absorption - This method is applicable for determining the concentration of many metals. The instrument is usually used only in a laboratory. It is adaptable to:
- 1 an automatic sampler
 - 2 automatic reagent additions
 - 3 automatic extractions

- D Photometric Methods - With automated addition of reagents, the end product can be measured with flow through cells in:

- 1 Visible range
- 2 Ultra violet range

- E Organic Carbon Analyzer - Manual injection of samples vs. automated sampling system is dependent on amount of suspended solids in samples.

IV PRESERVATIVES AND QUALITY CONTROL

- A Preservatives added to samples must be compatible with the automated method to be used. For example, sulfuric acid and mercury may interfere with colorimetric methods.

With automated methods, the preservative should be added to samples, standards and control blanks.

- B Routine standardization of automated instrument - After every 10 or 20 samples:

- 1 Check baseline with a control blank
- 2 Check sensitivity with a standard

The laboratory should also establish a program for routine interlaboratory and intralaboratory quality control.

V AUTOMATED WET-CHEMICAL SYSTEMS

Many chemical parameters for water quality are defined by the conditions in the chemical analytical methodology of Standard Methods, e.g. C.O.D., chlorine, phenolics and TKN digestion. All new automated methods should be compared to the manual Standard Method for accuracy.

- A Robot Chemist - individual sample and reaction vessel, syringe type measurement of reagents, micro-switches and valves, complex mechanics. Many are not very well accepted by chemists.

B Mecolab - individual sample and reaction containers; maximum 15 samples without manual change

C Technicon AutoAnalyzer - a continuous flow through system propelled by depression of various inside diameters of tygon tubing using constant speed rollers. The modular system is capable of almost all manual chemical techniques, such as sampling (up to 200 samples), filtration, dilution, addition of reagents, mixing, heating, dialysis, steam distillation, extraction with an immiscible solvent and digestion. The concentration of the end products is measured with a photometer or fluorometer and recorded. The versatility of the AutoAnalyzer enables the automation of almost any manual procedure.

The success of each automated procedure is primarily dependent on the manifold design. Effort should be made to achieve the maximum rate of each chemical reaction by determining the optimum conditions of time, temperature, pH and reagent concentration. When this is obtained, there is generally an increase in sensitivity, thus requiring a smaller aliquot of sample with less chance of interfering ions, a shorter flow stream through the system which will produce a faster response and wash out (shorter sampling time) and smoother, steadier plateaus.

The Technicon AutoAnalyzer is now internationally used for automating wet-chemical procedures in the laboratory. The Analytical Quality Control Laboratory of EPA includes Technicon procedures in the manual⁽⁹⁾ of methods for Office of Water Programs laboratories.

VI CONTINUOUS MONITORING SYSTEM

A Several criteria or objectives have been established for using the AutoAnalyzer for continuous monitoring of a stream or a treatment plant influent or effluent:

- 1 Capability of continuous operation for one week with a minimum amount of manual manipulation (less than 4 man hours per week).
- 2 At least a $\pm 5\%$ accuracy at the 50% (0.30 absorbance) absorption concentration.
- 3 Reagents and base line should remain stable for a week.
- 4 Reagent consumption should be less than 4 liters per week.
- 5 There should be a minimum distance of reaction-flow stream for a fast response and equilibrium of the system.
- 6 It is desirable that colorimetric chemistry obeys Beer's Law for rapid calculations.

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TABLE 1

INSTRUMENTAL METHODS FOR ANALYSIS OF WATER QUALITY PARAMETERS

PARAMETER	SENSOR	MODE OF MEASUREMENT	USES*	EVALUATION
TEMPERATURE	probe	thermocouple	P, F, L, C	excellent
pH	electrode	potentiometric	P, F, L, C	good
CONDUCTIVITY	electrode	conductivity	P, F, L, C	good
SOLAR RADIATION	photocell	photometric	P, F, L, C	excellent
DISSOLVED OXYGEN	electrode	polarographic	P, F, L, C	good
CHLORIDE	electrode	conductivity	P, F, L, C	fair
	wet-chemical	potentiometric photometric	F, L, C	—
TURBIDITY	photocell (scatter)	photometric	P, F, L, C	good
COLOR	photocell (absorbance)	photometric	F, L, C	good
SUSPENDED SOLIDS	photocell	photometric	—	—
ALKALINITY	wet-chemical	potentiometric	L	—
	wet-chemical	photometric	L	—
ACIDITY	wet-chemical	potentiometric	L	—
	wet-chemical	photometric	L	—
CALCIUM	electrode	potentiometric	P, L	—
	flame	atomic absorption	L	—
	wet-chemical	photometric	L	—
MAGNESIUM	flame	atomic absorption	L	—
	wet-chemical	photometric	L	—
AMMONIA	electrode	potentiometric	P, L, C(?)	—
	wet-chemical	photometric	P, L, C(?)	good
NITRITE	wet-chemical	photometric	P, L, C(?)	good
NITRATE	probe	potentiometric	—	—
	wet-chemical	photometric	P, L, C	good
PHOSPHORUS	wet-chemical	photometric	P, L, C	good
SULFATE	wet-chemical	photometric	L	—
SULFIDE	wet-chemical	photometric	L	—

TABLE 1 CONTINUED

INSTRUMENTAL METHODS FOR ANALYSIS OF WATER QUALITY PARAMETERS

PARAMETER	SENSOR	MODE OF MEASUREMENT	USES*	EVALUATION
TOTAL ORGANIC CARBON	wet-chemical	infrared	L	—
	wet-chemical	flame ionization	L	—
CYANIDE	electrode	potentiometric	P	—
	wet-chemical	photometric	P, L, C(?)	—
PHENOLICS	wet-chemical	photometric	—	—
SPECIFIC ORGANICS	gas-chromatography	electron-capture	L	—
	liquid-chromatography	photometric	L	—
BACTERIOLOGICAL	wet-enzymatic	photometric	—	—
	wet-fluorescence	fluorometric	—	—

*F-Field, monitoring streams

P-Treatment Plants, monitoring influent or effluent

L-Laboratory, with individual or automated sampling system

C-Continuous operation, for monitoring streams or treatment plants for operational control.

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This outline was prepared by Lawrence J. Kamphake, Research Chemist, Waste Identification & Analysis Program, AWTIL, RATWRC, NERC, EPA, Cincinnati, OH 45268.

AERIAL RECONNAISSANCE IN POLLUTION SURVEILLANCE

I INTRODUCTION

A Definition

The word "reconnaissance" is derived from the word "reconnoiter" which means to conduct a preliminary examination or survey. Its earlier applications to engineering and military requirements has been expanded to include photomapping and interpretations of natural resources. Aerial reconnaissance can be defined as "airborne examination or survey procedures performed by heavier-than-air craft, lighter-than-air craft, or earth orbiting satellites."

B Types of Aerial Reconnaissance

1 Visual

Examination of the flight path by a human observer with no provision for permanent recording for later study. This form of aerial observation has limited use but in many cases can complement the other forms.

2 Image forming sensor

Image recording of the covered flight path where maximum advantage can be made of image interpretation techniques. Image forming sensors includes such instruments as cameras, infrared scanners, and radar.

3 Nonimage forming sensors

Nonimage forming sensors include such devices as oscilloscopes, strip charts, and dial indicators which directly indicate parameter differentials as received by the sensing elements from the target scan.

4 Combination image forming and nonimage sensors

It is advantageous at times to combine both of these techniques in order to rapidly interpret flight path imagery.

This advantage is shown in Figure 1 where a heated effluent discharge is traced to a canal by infrared imagery and indicated by the "lighter" plume which parallels the shoreline. The oscilloscope phototracing indicates the temperature differential of the discharge point as compared to the offshore bay area.

C Detected images can be identified by one or more of the following criteria.

- 1 Size of image
- 2 Shape of image
- 3 Tonal qualities of image
- 4 Image profile shadowing
- 5 Location of image
- 6 Texture patterns of grouped images
- 7 Spatial relationship of image to surrounding bodies

D Selection of Remote Sensing Method

- 1 A knowledge of the electromagnetic spectrum (Figure 2) is important in order to select the most advantageous method of remote sensing. It will be noted that there are transmissibility and sensitivity limits as well as existing film limits with respect to equipment wave length patterns.
- 2 Remote sensing methods can be categorized into two areas.
 - a Source active: Utilization of an instrument which is capable of emitting a source of energy which is transmitted to the target area and emanations are received whose characteristics are dependent upon the nature of the specific target.

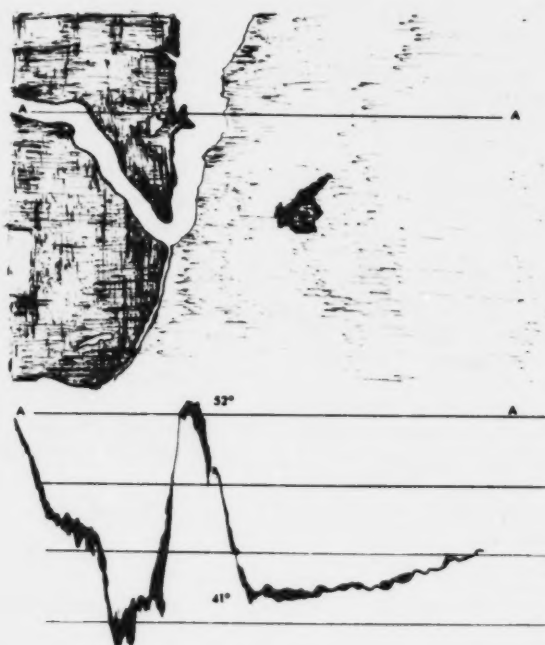


FIGURE 1

An example of such a device is the Radar set (Figure 3) or special Infrared Emitters both of which are capable of being utilized in total darkness.

- b Source passive: Utilization of instrumentation which is only capable of receiving target area emanations. Such remote sensing instruments, therefore, do not transmit artificial energy sources but depend upon the sun as a source of energy which is selectively received and individually reflected by each object in the target area. Aerial photography and most of the remote sensing is by this method and the included regions of the electromagnetic spectrum are the upper portions of the UV band to the near infrared region.
- 3 Selection of desired wavelength is dependent upon the optimum emission capability of the instrument which has the shortest wavelength possible to sharply differentiate small objects while being long enough to preclude excessive

energy "scattering" and yet not long enough so that photographic recording is impossible.

Utilization of camera systems (Multi-band) exposing discrete bands of the photographic spectrum simultaneously have allowed the photo-interpreter to make discriminations which are not possible with single bands of black-and-white photography. The interpreter may use various projection devices which allow the viewing of one, two, or more images superimposed for a more sophisticated analysis capability.

II TECHNIQUES

A Radar(RA dio De tect ion And Rang ing)

Radar is particularly useful for discerning certain types of vegetation which may not appear in the best visual quality in Panchromatic Color Film (sensitive to the

ELECTROMAGNETIC SPECTRUM

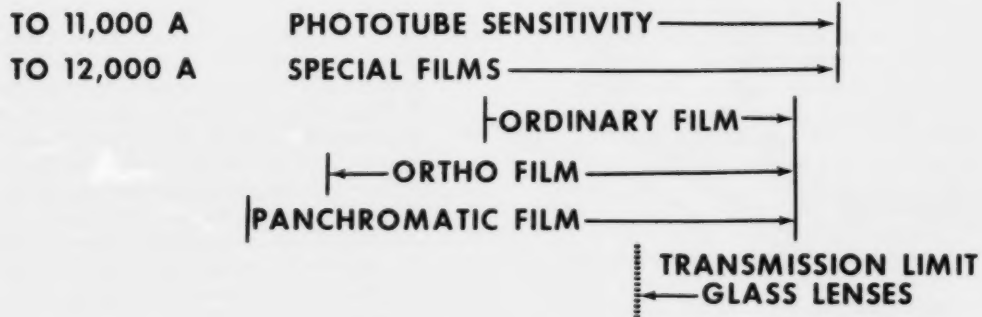
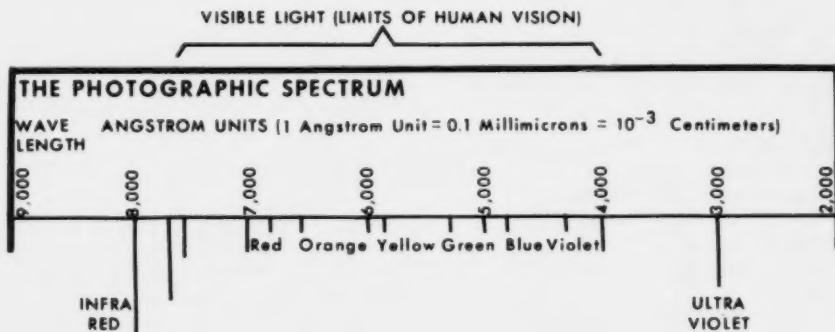
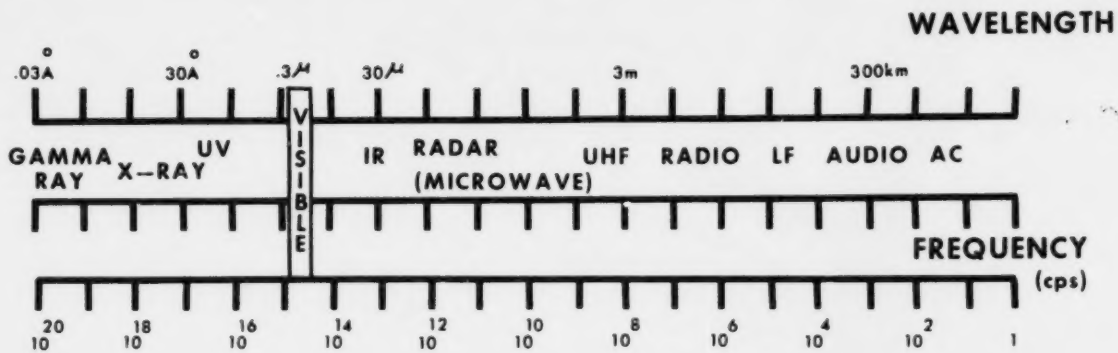


FIGURE 2

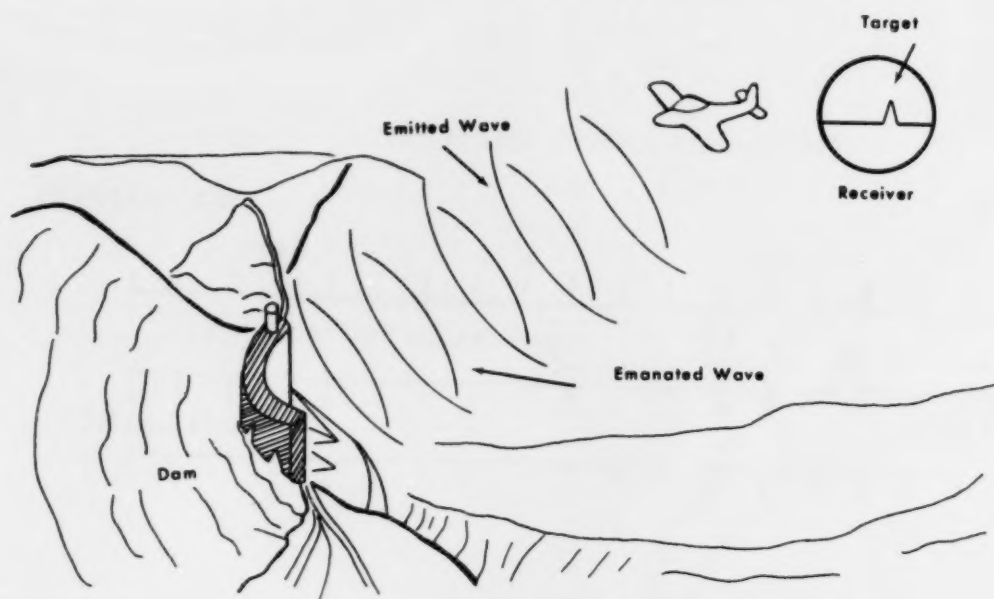


FIGURE 3

entire visible spectrum) and also has all-weather and around-the-clock capabilities. Radar in the longer wavelength ranges is capable of penetrating dense vegetative cover and this advantage has been found to be useful in detecting drainage networks and geological features.

B Conventional Photography

Conventional photography covers the entire range of the visible spectrum and employs both black-and-white film and color film. Some of the special films are capable of photographing a portion of the infrared band (the "near" infrared region) and this capability is useful in special situations.

1 Direct photomapping and panoramic scan

Figure 4 illustrated the manner in which direct photomapping differs from the panoramic scanning technique and it can be seen that continual attached mapping scans can be made of all or portions of the flight path to be analyzed.

2 Multiband photography

Multiband photography is, as its name implies, the synchronized photography of up to nine separate photographs of the same target area each of which is photographed in a different wavelength. A distinct advantage of this method is that, during photo analysis, distinct anomalies may be evident between objects appearing in the different bands and further investigation may be warranted. An example of this is shown in Figure 5 where it was observed that an anomaly in vegetative growth was apparent in the Infrared wavelength while not being apparent in the other visual spectral ranges. Further investigations proved that gravel washings in slug intervals had affected normal growth of the streamside vegetation.

3 Infrared photography

Infrared photography has been found useful in depicting certain hydrological features. Within the photographic

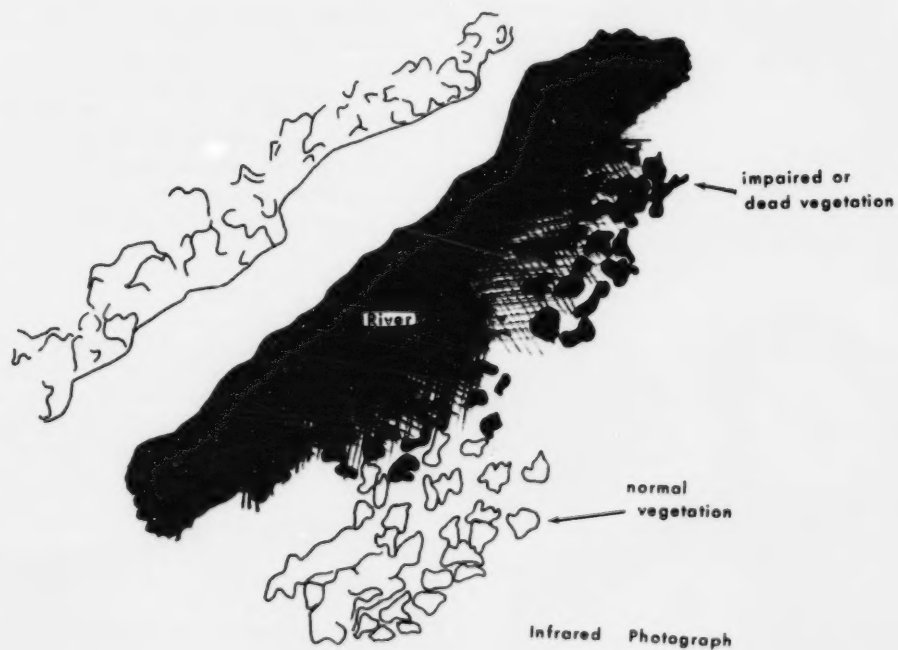
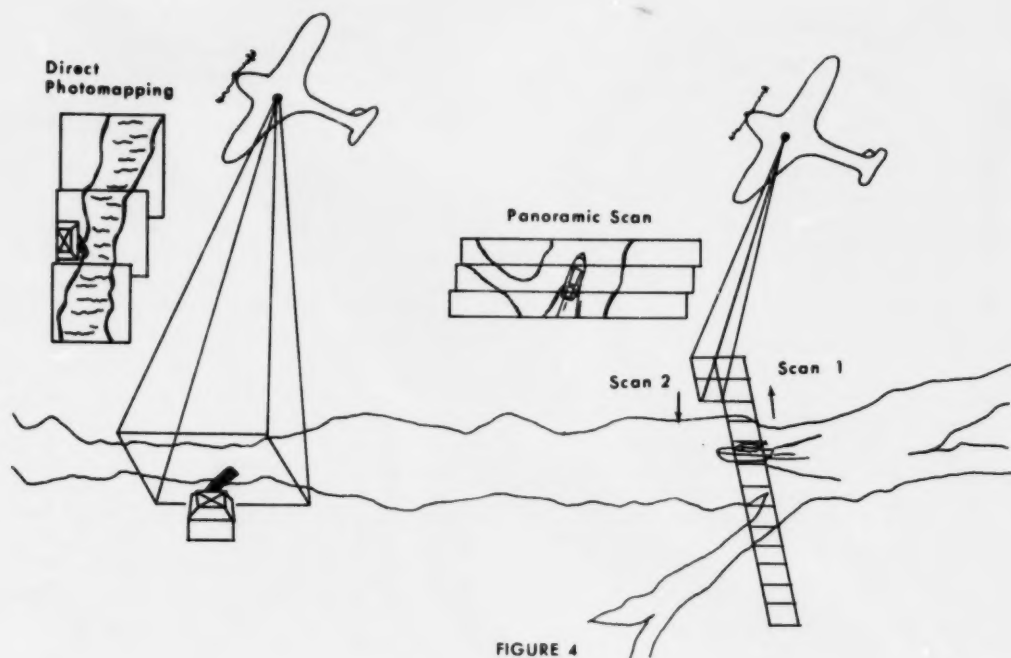


FIGURE 5

ranges it is not a heat-measuring tool and therefore cannot detect thermal anomalies within bodies of water but instead sharply delineates shoreline and tributary features and has haze penetrating qualities. The blues appear much darker by infrared photography and the reds, greens, and yellows will show up much lighter when compared to a panchromatic color photograph.

4 Gamma Ray Spectrometer

This device functions in the very short wavelengths and has been found to have excellent detecting capabilities for radioactivity. Such a device would serve as an excellent means of searching for radioactive waste spills which have occurred either accidentally or intentionally.

C Infrared Radiometry

Infrared imagery is different than infrared photography since the tone of the imagery is directly related to the infrared radiation emitted from the target area. This type of scanner converts the normally unphotographic IR band to a thermal imagery. An optical-mechanical device is necessary since the normal thermal energy emitted by the camera itself will influence radiations received from the target area and this elimination of detector internal radiation is accomplished by miniturization and application of extremely low temperatures. One example of the usefulness of infrared imagery is shown in Figure 6 where the dark areas along the shoreline were found to be caused by cold water infiltration by a previously unknown source. Such a finding could be indicative, for example, of a new untapped freshwater source or a polluting influence from a remote source. This cold water infiltration was not evident by normal visual spectrum photography and the IR photograph would only indicate a more distinct shoreline.

D Automatic Analysis

In the analysis of large numbers of prints use can be made of photoelectric scanners

wherein automatic recording of degree of brightness can be recorded on a tape printout. An example of this procedure is shown in Figure 7 where each symbol has a significance relating to "tone signature." The "D" zones are the darker areas while the other symbols relate to lighter zones. In the center the clear area is a river tributary. Future predictions are that automatic encoding can be channeled to computers wherein decisions can be made, for instance, in water management policies.

E Stereoscopic Analysis

The principles and methods developed during World War II for the stereoscopic analysis of aerial photographs has been a continuing and expanding science. Its principles are based upon the fact that the human eyes are normally spaced about 65 millimeters apart and are capable of compositing, via the optic nerve, the two separate and distinct images viewed by each orbit. Aerial stereoscopic analysis depends upon the placing of two photographs precise distances apart with precise overlapping as required by normal human visual acuity. With the aid of special stereoscopic viewing lenses the viewer is able, in most cases, to composite both of the photographs in the same manner as with normal vision and thus a sense of depth can be imparted materially enhancing the analyzing of the terrain.

III APPLICATIONS

Aerial reconnaissance in pollution surveillance can be accomplished in a variety of ways and the method chosen usually is dictated by costs involved and what is desired with regard to immediate or potential information.

A Visual Observations

The simplest and least expensive method is to visually observe the study area and this can be useful in determining sampling sites, locating previously undetermined tributaries, pinpointing visual gross

[illegible]

37-7

pollutant effluents, etc. To augment these observations it is usually better to have a permanent recording of the survey area in the form of photographic or imagery recordings.

B Photographic Techniques

Photographic evidence, either color, black-and-white, or special film, can be recorded in various attitudes or positions of the aircraft in order to make best use of interpretation techniques such as stereoscopic analysis.

1 Current patterns and flow velocity can be ascertained by a method developed by the U.S. Coast and Geodetic Survey whereby powdered aluminum is surface distributed to a wide area and subsequent photographic patterns can be analyzed. Flowing bodies can either appear as "depressions" or "elevations" depending upon direction of flow with relation to the photographing aircraft and from these "parallax" anomalies the velocity can be determined.

2 Photographic IR has found applications in the areas of detecting sharp delineations of shorelines and tributaries especially when haze is prevalent.

3 The work of Strandberg¹ concludes that "In aerial photographs, oxygen-deficient water frequently appears black, or at least darker in tone and this appearance may be caused by the incomplete reduction of wastes by anaerobic bacteria." Thus his black-and-white photographs of waterbodies with low values of dissolved oxygen (DO) following a source of waste discharge are characterized by a plume discharge followed downstream by dark water tones.

Further values ascribed to photography in this manual include color photographs dealing with fish kills where the extent of damage can be better assessed and the use of "false" color infrared

photography where algal masses can be differentiated and, again, the low DO concentration is manifested by a color anomaly.

C Imagery Techniques

1 Utilization of the "mid" and "far" infrared wavelengths in the form of "imagery" has been found useful in analyzing for thermal pollution and its concomitant circulation and diffusion patterns for which "thermographs" can be described for large water bodies.² It is also possible to establish naturally occurring patterns of seasonal and diurnal thermal ranges for surface waters.

2 It has been ascertained, in principle, that it is possible to identify pollutants by study of spectral emissivity characteristics for particular wavelengths and this can be of value, for instance, when one is searching for a particular pollutant and the emissivity characteristics for the waterbody can be compared with known patterns for the specific pollutant.²

A hypothetical situation is plotted in Figure 8 where spectrometric values of polluted water discharge are compared to a "normal" pattern of the water body.

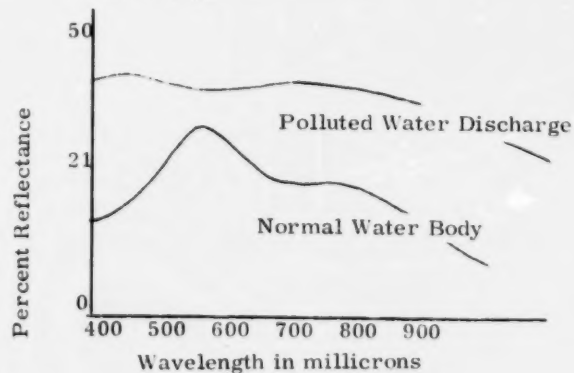


Figure 8

- 3 Utilization of radar and orbiting satellite platforms can produce imagery of great usefulness. The radar technique can precisely delineate shoreline characteristics without vegetative interferences and thus can thoroughly image drainage basins. Satellite imagery and photography can obtain the same information but on a much broader scale and with great rapidity. Recent lines of thought have postulated that this orbiting scanner can be fitted with computing devices to render decisions upon analysis of its constant data collection.³ Such decisions can be of immeasurable aid to the water conservationist who may require long range predictions based upon analysis of whole river basins.

IV SUMMARY

Development of aerial reconnaissance in pollution surveillance has proceeded in the areas of technique and interpretation. With regard to the techniques available most of the earlier problems have been overcome to a degree where available material is amenable to analysis. Such early problems as pitch and yaw of the aircraft, cloud cover, imagery instrumentation, etc., has been overcome to a large degree by available engineering skills and the need for improvement due to military needs. In the area of interpretation, however, much has yet to be accomplished to develop this tool as an extension of laboratory analysis which it will augment rather than replace. It is highly possible

that remote sensing will take the final evolutionary form of fully automatic computerized operations. A variety of remote sensing devices can be envisioned to collectively contribute to a final printout of management decisions.

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This outline was prepared by R. Russomanno, Microbiologist, National Training Center, DTTB, MDS, WPO, EPA, Cincinnati, OH 45268.

CASE PREPARATION AND COURTROOM PROCEDURE

I TYPES OF PROCEEDINGS IN WHICH WATER QUALITY EVIDENCE MAY BE USED

A Administrative Proceedings

1 Rule making

- a Setting up of regulations having general application, e.g., stream classifications and implementation plan target dates
- b Factors of safety and absolute prohibitions may be appropriate

2 Adjudications

- a Determinations by agency having expertise with respect to particular discharge or discharger, e.g., approval of plans and specs and time schedule of a particular discharger

B Court Actions

1 Civil in behalf of state or federal government

- a Actions to compel action or suspension of action - nuisance, health hazard, etc., --including court action following federal conference --hearing procedure
- b Violations of Water Quality Standards
- c Violations of Effluent Standards or discharge permits
- d Tort or contract actions relating to design and/or operation of treatment facilities

2 Criminal (dependent on content of applicable statutes)

- a Discharge of specific materials

b Discharges from specific industries

c Littering

d Discharges harmful to fish and/or crustaceans

e Discharges harmful to specific types of receiving waters

f Discharges of poisons

NOTE--In some of these situations doing the act may constitute the violation; in others proof of intent or knowledge of effects may also have to be proved.

3 Private actions for damages or to compel action

- a Alleged harm to plaintiff, e.g., pollution of stream killing animals

C Procedural Matters

- 1 See Attached sheet "Administrative and Court Proceedings" on Burden of proof, fact finding, and methods of presentation of evidence.

D Classes of Evidence - General Rules

1 Facts - direct

- a The material was floating from the outfall.

2 Derived values - expert testimony - test results and/or opinion as to effects

- a The D.O. was zero; the waterway was polluted; the plant can be built in 6 months.

3 Hearsay

- a Joe told me

4 Relevancy

5 Admissibility vs. weight

- a Even if admissible, the weight to be given is up to fact finder--credibility.

E Admissibility of Results of Sampling and Testing (Numbers)

1 Sampling

- a Chain of custody
- b Tags, etc.
- c Containers
- d Place and time
- e Retention of samples (Proving that the sample represents what is at issue in the action (relevancy), that there has been no opportunity for tampering; and availability of portions for analysis by other side (non-transitory criteria)).

2 Analysis

- a Who performed (Can identity of each participant be shown?)
- b Admission through supervisor - custodian
- c Scientific acceptance of method. Is there a particular method required to be used by the agency?
- d Propriety of conduct
- e Retention of bench cards and other indicia of results. (Your attorney can make arrangements to substitute copies for originals).

3 Tests

- a Comparison with actual conditions
- b Mathematical models - how can a computer be cross-examined?

F Admissibility of Expert Opinion on Causes and Effects

- 1 Who has special knowledge - and of what particular areas?
- 2 Indicators
- 3 Significance of numerical determinations or observations
- 4 Consistency with own prior publications and testimony
- 5 Have underlying facts been or need to be proved--first hand information of this and/or comparable situations.
- 6 Use of treatises

G Conduct on the Witness Stand

1 General

- a On direct - know what counsel will ask and let him know generally what you will answer, but don't make it sound rehearsed.
- b Use layman's language to extent possible.
- c Listen to question and answer it to best of your ability.
- d Speak so that court reporter, judge, jury, and counsel can hear you.
- e Speak in language that will be understood; don't talk down.
- f Answer only what you are asked --don't volunteer; however, answer with precision.
- g There is nothing wrong with asking to have a question repeated or rephrased.
- h There is nothing wrong with saying that you consulted with your attorney before you testified, but beware of the question "Did Mr. X tell you what to say?"

- i There is nothing wrong with thinking out your answer before responding.
 - j You are not expected to know all the answers--if you do not know, admit it.
 - k Don't attempt to answer questions outside your area of personal knowledge (hearsay) or beyond your expertise. (Your may be an expert on conducting laboratory tests, but not on epidemiological inferences from results).
 - l Don't try to answer before the judge rules on objection.
 - m Show that you are an impartial dispenser of information and/or opinion, not a protagonist.
 - n Don't be afraid to admit what may appear to be damaging.
- 2 If you are testifying as an expert:
- a Establish qualifications -- give information relevant to your area of expertise -- educational (including this course?), work, publications, number of times you have testified previously.
 - b Differentiate between physical facts (measurements and observations) and opinion (derived values).
 - c Be prepared to discuss theory (including assumptions) instruments used, techniques(including choice of a particular technique), physical limitations and errors, interferences.
 - d If experiments were conducted, be able to justify both as to theory and relevancy to this litigation.
 - e If you're being paid to testify, admit it.
- 3 Scientific personnel as advisers to counsel:
- a Review and refamiliarize self with materials before you discuss with your attorney.
 - b Be in a position to present all facts known to you simply and concisely: Who, What, When, Where, and Why, How.
 - c Don't overlook facts and/or test results because you don't think they're important. Let attorney decide what he needs.
 - d Use of standard report forms
 - e Ability to recommend additional witnesses with needed specialized knowledge
 - f Ability to aid in cross-examination of other side's experts and reconcile opinions and/or results
 - g Be candid - sometimes better not to start a lawsuit or accept a settlement than lose in the end.
- H Non-Verbal Presentation of Evidence
- 1 Exhibits - including photographs
 - 2 Summaries
 - 3 Business and/or government records
 - a Prepared contemporaneously and in usual course of activities
 - 4 Pre-prepared direct examination
 - a Usually limited to actions before ICC, FPC, and other federal agencies.
- I Criminal Procedure
- 1 Privilege Against Self Incrimination (available only to persons)
 - a Warning and suspects
 - b Effect of duty to report spills

Case Preparation and Courtroom Procedure

- c Effect of duty to obtain license or permit and/or furnish operating reports
 - d Immunity from prosecution
- 2 Double Jeopardy

- 3 Unreasonable search and seizure
- a Available to persons and corporations
- 4 Procedures and need for arrest and search warrants -- possible cause

This outline was prepared by David I. Shedroff, Enforcement Analyst, Office of Enforcement and General Counsel, Cincinnati Field Investigations Center, 5555 Ridge Avenue, Cincinnati, OH 45268.

Administrative & Court Proceedings,
and Excerpts from Revised Draft of
Proposed Rules of Evidence for the
United States Courts can be found on the
following pages.

ADMINISTRATIVE & COURT PROCEEDINGS

<u>Court or Agency</u>	<u>Fact Finder</u>	<u>Burden of Proof</u>	<u>Comments</u>
State Pollution Control Agency	Agency	As per statute - usually weight of evidence.	Hearing may be conducted by hearing examiner, agency member, or full agency. Appeal may be on facts and law or law alone, depending on statute.
Rule making-adjudication			
Federal Water Pollution Control Act			
Conference	Head of agency		Reports acceptable.
Hearing	Hearing Board		Specific testimony.
Court	Judge		Uses prior material, and may take additional testimony.
Court			
Civil Case -- - for money only	Judge or jury	Weight of evidence	
- injunction			
preliminary or temporary	Judge	Must show immediate harm or danger.	Must also show likelihood of success at final hearing - bond required for non-government plaintiff.
permanent	Judge	Usually clear and convincing.	"Balance Equities"
- administrative appeal	Judge - whether "arbitrary and capricious" or substantial evidence.		Sometimes have complete new trial.
Criminal case includes penalties	Jury unless waived.	Beyond reasonable doubt.	Proof of intent may be required.

Excerpts from Revised Draft of Proposed
RULES OF EVIDENCE FOR THE UNITED STATES COURTS

GENERAL PROCEDURES

Rule 102.

PURPOSE AND CONSTRUCTION

These rules shall be construed to secure fairness in administration, elimination of unjustifiable expense and delay, and promotion of growth and development of the law of evidence to the end that the truth may be ascertained and proceedings justly determined.

Rule 101.

PRELIMINARY QUESTIONS

(a) Questions of Admissibility Generally. Preliminary questions concerning the qualification of a person to be a witness, the existence of a privilege, or the admissibility of evidence shall be determined by the judge, subject to the provisions of subdivision (b). In making his determination he is not bound by the rules of evidence except those with respect to privileges.

(b) Relevancy Conditioned on Fact. When the relevancy of evidence depends upon the fulfillment of a condition of fact, the judge shall admit it upon, or subject to, the introduction of evidence sufficient to support a finding of the fulfillment of the condition.

Rule 615.

EXCLUSION OF WITNESSES

At the request of a party the judge shall order witnesses excluded so that they cannot hear the testimony of other witnesses, and he may make the order of his own motion. This rule does not authorize exclusion of (1) a party who is a natural person, or (2) an officer or employee of a party which is not a natural person designated as its representative by its attorney, or (3) a person whose presence is shown by a party to be essential to the presentation of his cause.

Rule 611.

MODE AND ORDER OF INTERROGATION AND PRESENTATION

(a) Control by Judge. The judge may exercise reasonable control over the mode and order of interrogating witnesses and presenting evidence so as to (1) make the interrogation and presentation effective for the ascertainment of the truth, (2) avoid needless consumption of time, and (3) protect witnesses from harassment or undue embarrassment.

(b) Scope of Cross-Examination. A witness may be cross-examined on any matter relevant to any issue in the case, including credibility. In the interests of justice, the judge may limit cross-examination with respect to matters not testified to on direct examination.

Rule 613.

PRIOR STATEMENTS OF WITNESSES

(a) Examining Witness Concerning Prior Statement. In examining a witness concerning a prior statement made by him, whether written or not, the statement need not be shown or its contents disclosed to him at that time, but on request the same shall be shown or disclosed to opposing counsel.

JUDICIAL NOTICE

Rule 201.

JUDICIAL NOTICE OF ADJUDICATIVE FACTS

(b) Kinds of Facts. A judicially noticed fact must be one not subject to reasonable dispute in that it is either (1) generally known within the territorial jurisdiction of the trial court or (2) capable of accurate and ready determination by resort to sources whose accuracy cannot reasonably be questioned.

(g) Instructing Jury. The judge shall instruct the jury to accept as established any facts judicially noticed.

RELEVANCE

Rule 401.

DEFINITION OF "RELEVANT EVIDENCE"

"Relevant evidence" means evidence having any tendency to make the existence of any fact that is of consequence to the determination of the action more probable or less probable than it would be without the evidence.

Rule 402.

RELEVANT EVIDENCE GENERALLY ADMISSIBLE;
IRRELEVANT EVIDENCE INADMISSIBLE

All relevant evidence is admissible, except as otherwise provided by these rules, by other rules adopted by the Supreme Court, by Act of Congress, or by the Constitution of the United States. Evidence which is not relevant is not admissible.

COMPETENCY OF WITNESSES

Rule 601.

GENERAL RULE OF COMPETENCY

Every person is competent to be a witness except as otherwise provided in these rules.

Rule 602.

LACK OF PERSONAL KNOWLEDGE

A witness may not testify to a matter unless evidence is introduced sufficient to support a finding that he has personal knowledge of the matter. Evidence to prove personal knowledge may, but need not, consist of the testimony of the witness himself. This rule is subject to the provisions of Rule 703, relating to opinion testimony by expert witnesses.

EXPERT TESTIMONY

Rule 702.

TESTIMONY BY EXPERTS

If scientific, technical, or other specialized knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training, or education, may testify thereto in the form of an opinion or otherwise.

Rule 703.

BASES OF OPINION TESTIMONY BY EXPERTS

The facts or data in the particular case upon which an expert bases an opinion or inference may be those perceived by or made known to him at or before the hearing. If of a type reasonably relied upon by experts in the particular field in forming opinions or inferences upon the subject, the facts or data need not be admissible in evidence.

Rule 705.

DISCLOSURE OF FACTS OR DATA UNDERLYING EXPERT OPINION

The expert may testify in terms of opinion or inference and give his reasons therefore without prior disclosure of the underlying facts or data, unless the judge requires otherwise. The expert may in any event be required to disclose the underlying facts or data on cross-examination.

Rule 706.

COURT APPOINTED EXPERTS

(a) Appointment. The judge may on his own motion or on the motion of any party enter an order to show cause why expert witnesses should not be appointed, and may request the parties to submit nominations. The judge may appoint any expert witnesses agreed upon by the parties, and may appoint witnesses of his own selection. An expert witness shall not be appointed by the judge unless he consents to act. A witness so appointed shall be informed of his duties by the judge in writing, a copy of which shall be filed with the clerk, or at a conference in which the parties shall have opportunity to participate. A witness so appointed shall advise the parties of his findings, if any; his deposition may be taken by any party; and he may be called to testify by the judge or any party. He shall be subject to cross-examination by each party, including a party calling him as a witness.

HEARSAY

Rule 801.

DEFINITIONS

The following definitions apply under this Article:

- (a) Statement. A "statement" is (1) an oral or written assertion or (2) nonverbal conduct of a person, if it is intended by him as an assertion.
- (b) Declarant. A "declarant" is a person who makes a statement.
- (c) Hearsay. "Hearsay" is a statement, other than one made by the declarant while testifying at the trial or hearing, offered in evidence to prove the truth of the matter asserted.

Rule 802.

HEARSAY RULE

Hearsay is not admissible except as provided by these rules or by other rules adopted by the Supreme Court or by Act of Congress.

Rule 803.

HEARSAY EXCEPTIONS: AVAILABILITY OF DECLARANT IMMATERIAL

The following are not excluded by the hearsay rule, even though the declarant is available as a witness:

- (5) Recorded Recollection. A memorandum or record concerning a matter about which a witness once had knowledge but now has insufficient recollection to enable him to testify fully and accurately, shown to have been made when the matter was fresh in his memory and to reflect that knowledge correctly. If admitted, the memorandum or record may be read into evidence but may not itself be received as an exhibit unless offered by an adverse party.
- (6) Records of Regularly Conducted Activity. A memorandum, report, record, or data compilation, in any form, of acts, events, conditions, opinions, or diagnoses, made at or near the time by, or from information transmitted by, a person with knowledge, all in the course of a regularly conducted activity, as shown by the testimony of the custodian or other qualified witness, unless the sources of information or other circumstances indicate lack of trustworthiness.
- (18) Learned Treatises. To the extent called to the attention of an expert witness upon cross-examination or relied upon by him in direct examination, statements contained in published treatises, periodicals, or pamphlets on a subject of history, medicine, or other science or art, established as a reliable authority by the testimony or admission of the witness or by other expert testimony or by judicial notice. If admitted, the statements may be read into evidence but may not be received as exhibits.

IDENTIFICATION OF PERSONS AND SAMPLES

Rule 901.

REQUIREMENT OF AUTHENTICATION OR IDENTIFICATION

(a) General Provision. The requirement of authentication or identification as a condition precedent to admissibility is satisfied by evidence sufficient to support a finding that the matter in question is what its proponent claims.

(b) Illustrations. By way of illustration only, and not by way of limitation, the following are examples of authentication or identification conforming with the requirements of this rule:

(1) Testimony of Witness with Knowledge. Testimony that a matter is what it is claimed to be.

(3) Comparison by Trier or Expert Witness. Comparison by the trier of fact or by expert witnesses with specimens which have been authenticated.

(9) Process or System. Evidence describing a process or system used to produce a result and showing that the process or system produces accurate result.

ADMISSIBILITY AND PROOF OF SPECIAL MATTERS

Rule 406.

HABIT; ROUTINE PRACTICE

(a) Admissibility. Evidence of the habit of a person or of the routine practice of an organization, whether corroborated or not and regardless of the presence of eye-witnesses, is relevant to prove that the conduct of the person or organization on a particular occasion was in conformity with the habit or routine practice.

(b) Method of Proof. Habit or routine practice may be proved by testimony in the form of an opinion or by specific instances of conduct sufficient in number to warrant a finding that the habit existed or that the practice was routine.

Rule 612

WRITING USED TO REFRESH MEMORY

If a witness uses a writing to refresh his memory, either before or while testifying, an adverse party is entitled to have it produced at the hearing, to inspect it, to cross-examine the witness thereon, and to introduce in evidence those portions which relate to the testimony of the witness.

Rule 1006.

SUMMARIES

The contents of voluminous writings, recordings, or photographs which cannot conveniently be examined in court may be presented in the form of a chart, summary, or calculation. The originals, or duplicates, shall be made available for examination or copying, or both, by other parties at a reasonable time and place. The judge may order that they be produced in court.

IX. LABORATORY PROCEDURES

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LABORATORY PROCEDURE FOR TOTAL ALKALINITY

I METHOD SUMMARY

A In the current methods manual⁽¹⁾ of the Environmental Protection Agency, the following method is specified for use in Office of Water Programs laboratories. The sample is titrated with 0.02N hydrochloric or sulfuric acid to a final pH of 4.5, the end point being determined electrometrically.

- 1 The sample must not be filtered, diluted, concentrated or altered in any way.
- 2 Results are reported as mgCaCO₃/liter.
- 3 The procedure can be found in Standard Methods⁽²⁾ and ASTM Book of Standards.⁽³⁾

B The procedure as given in the above references has been adapted for this laboratory session to accommodate group participation and to accomplish instructional objectives.

II DISCUSSION

In this manual, outline 8 considers the relationships among the 3 forms of alkalinity commonly found in water. Refer to the graphs in outline 8 for the following:

For Case 1, (OH)⁻ alkalinity only, pH values change very gradually until the approach of pH 9. Then an abrupt change occurs and the addition of a few ml of titrant causes the value to change rapidly to about pH 4.

For Case 2, (CO₃)⁼ alkalinity only, there is a gradual change of pH values as the acid titrant is added. Around pH 9 there is an abrupt change of the scale reading. Around pH 6, the change is again gradual. Around pH 5, another rapid change occurs until pH 3 is reached.

A pH of 8.3 represents the average pH when (OH)⁻ has been totally neutralized and

(CO₃)⁼ has been half neutralized to (HCO₃)⁻. A pH of 4.5 represents the average pH occurring when (HCO₃)⁻ has been neutralized to CO₂ and H₂O. (See Case 3).

In order to obtain standardized data, the EPA method specifies stopping alkalinity titrations at pH 4.5. At this pH, any (OH)⁻, (CO₃)⁼ and (HCO₃)⁻ in the sample will be neutralized. Since all three of these alkalinity forms may be present, the results are expressed as total alkalinity.

It is useful to express total alkalinity as mg CaCO₃/liter.

III INSTRUCTIONAL OBJECTIVES

A You will use the adapted EPA method to determine total alkalinity by titrating 3 solutions of known alkalinities and one unknown to an end point of pH 4.5. You will then use the data to calculate the total alkalinity for each as mg CaCO₃/liter.

- 1 By slowly adding the HCl titrant to solutions of (OH)⁻, of (CO₃)⁼ and of (HCO₃)⁻ and observing the pH scale, the characterizing response of each alkalinity at different pH values can be observed.
- 2 Recording the volume of HCl used to reach pH 8.3 and then pH 4.5 will also illustrate the pH characteristics of the three forms of alkalinity.
- 3 Calculating the total alkalinity of these three solutions will familiarize you with the calculations for the determination.

B You will also do titrations on the same solutions using color indicators --- phenolphthalein (colorless at pH 8) and methyl orange (amber-orange at pH 4.6) --- in order to contrast values obtained by the pH determination.

- C Using experience gained in the lab session, you will estimate the types of alkalinity present in the unknown sample.

IV REAGENTS

- A Carbon Dioxide-Free Distilled Water
- B Anhydrous Sodium Carbonate (primary standard)
- C Hydrochloric Acid Titrant (0.02N)
- D Phenolphthalein
- E Methyl Orange

V STANDARDIZATION of the HYDROCHLORIC ACID TITRANT

- A Set the temperature reading on the pH meter dial to match the temperature of the buffer and sample solutions.
- B Standardize the pH meter against a reference buffer solution.
- C Weigh accurately 0.088 ± 0.001 g of the dried sodium carbonate and transfer it to a 500 ml conical flask.
- D Add 50 ml of water and swirl to dissolve the carbonate.
- E While stirring the solution, add the hydrochloric acid titrant from a 100 ml buret until a pH of 4.5 is attained.
- F Calculate the normality of the hydrochloric acid solution as follows:

$$A = \frac{B}{0.053 \times C}$$

A = normality of the hydrochloric acid

B = g of sodium carbonate used

C = ml of hydrochloric acid consumed

0.053 = millequivalent weight of Na_2CO_3

VI TITRATIONS to pH END POINTS

For each of the solutions marked Case 1 (OH^-), Case 2 (CO_3^{2-}) and Case 3 (HCO_3^-) and for the assigned unknown, A or B, the following procedure is to be followed. All data and the calculation results should be recorded on the Data Table provided.

A Calibrating the pH meter

- 1 Pour about 50 ml of the buffer solution into a small beaker.
- 2 Set the temperature reading on the pH meter dial to match the temperature of the reference buffer.
- 3 Immerse the electrode(s) into the buffer solution.
- 4 Turn the meter to "ON" (or "Read" or "pH").
- 5 Using the "Balance" (or "Asymm") knob, set the scale reading to 6.84.
- 6 Turn the meter to "Off" (or "Hold" or "Standby").
- 7 Remove electrode(s) from buffer and save this solution for possible later use.
- 8 Rinse electrode(s) with distilled water, then immerse them in a small beaker of distilled water until time to use.

B Prepare the buret by rinsing 3 times using about 15 ml HCl each time. Then fill the buret with HCl and adjust the volume so that the top of the liquid column is positioned for a reading.

C Titration Procedure:

- 1 Each titration will require up to 25 ml 0.02N HCl titrant. Refill the buret as needed before beginning each titration.
- 2 Pipet 50.0 ml of the solution to be used into a beaker.

- 3 Remove the pH electrode(s) from the beaker of distilled water and immerse electrode(s) in the solution to be titrated. The sample solution should cover the glass bulb(s).
- 4 Turn pH meter to "ON" (or "Read" or "pH").
- 5 On the Data Table, record the ml of HCl in the buret at the start of the titration.
- 6 Record the initial pH of the sample on the Data Table.
- 7 While gently swirling either the electrode or the sample in the beaker, slowly add the HCl titrant, observing the scale on the pH meter. Stop adding titrant when pH is about 8.3 and record the buret reading. (This step is not part of the EPA procedure. It is included here for instructional purposes).
- 8 Again gently swirling either the electrode or the beaker, continue slow addition of the HCl titrant, observing the meter scale. Be very cautious when the scale approaches pH 5.0. Stop titrating at pH 4.5. If a lower pH is inadvertently reached, note this on the Data Table.
- 9 On the Data Table, record the ml HCl remaining in the buret.
- 10 Set pH meter to "OFF" (or "Hold" or "Standby").
- 11 Rinse the pH electrode(s) with distilled water and immerse in the beaker of distilled water.
- 12 Empty the sample solution and rinse the beaker with distilled water.
- 13 Using the next solution, repeat this procedure (C, steps 1 through 12) until data has been recorded for the 3 knowns and the 1 unknown sample. Then proceed to part VII.

DATA TABLE
TITRATIONS TO pH END POINTS

	CASE 1 (OH)	CASE 2 (CO ₃)	CASE 3 (HCO ₃)	SAMPLE _____
Initial pH				
ml@pH 4.5				
ml@pH 8.3				
ml@Start				
TOTAL ml. HCl				
TOTAL ALKALINITY (mg CaCO ₃ /liter)				

VII TITRATIONS to COLOR END POINTS

If time permits, the following procedure should be used to titrate the 3 solutions of known constituents and the assigned unknown, A or B. At least, do one of the known solutions and the unknown used for the pH titration. All data and the calculation results should be recorded on the Data Table provided on page 39-4.

A Indicators Used

- 1 Phenolphthalein indicator is a deep pink around pH 10 and becomes colorless at pH 8. In the Data Table, this colorless end point is designated as P. End Pt.
- 2 Methyl orange indicator gives a yellow color to a colorless solution. At pH 4.6 (referred to as M. O. End Pt. on Data Table) it becomes amber-orange. This end point color requires practice to detect. If the solution acquires a pinkish cast, you are past the desired end point.

B Titration Procedure

- 1 Each titration will require up to 25 ml 0.02N HCl titrant. (If pH determinations have been done, refer to these results for approximations of HCL titrant to be used). Refill the buret as needed before beginning each titration.

Laboratory Procedure for Total Alkalinity

- 2 Pipet 50.0 ml of the solution to be used into a beaker.
- 3 Place a piece of white paper under the beaker.
- 4 Add 3 drops phenolphthalein indicator to the sample and swirl to mix.
- 5 On the Data Table record the ml of HCl in the buret at the start of the titration.
- 6 Using the known relationship of the color of phenolphthalein to different pH values, record your estimate of the initial pH of the sample on the Data Table.
- 7 If the solution remains colorless, record 0.0 for P. End Pt. and continue with step 9.

If there is pink color in the solution, slowly add HCl titrant while stirring the sample.

As the end point is approached, a lingering colorless region where the titrant first contacts the sample will be observed. Add the titrant very cautiously at this time, stirring the sample solution and allowing time for total mixing and contact before adding more HCl titrant.

The end point occurs when the entire solution is colorless. Stop adding the HCl titrant.

- 8 Record the ml of HCl in the buret for the P. End Pt. on the Data Table.
- 9 Add 3 drops of methyl orange indicator to the same sample solution.
- 10 If the sample solution becomes amber-orange, record 0.0 for M.O. End Pt. and continue at step 12.

If the solution becomes yellow, slowly add HCl titrant while stirring the sample.

As the end point is approached, a lingering amber-orange region where the titrant first contacts the sample will be observed. Add the titrant very cautiously at this time, stirring the sample solution and allowing time for total mixing and contact before adding more HCl titrant.

The end point occurs when the entire solution is amber orange. Stop adding the HCl titrant. (If the solution develops a pinkish cast, you are past the desired end point and should note this on the Data Table).

- 11 Record the ml of HCl in the buret for the M.O. End Pt. on the Data Table.
- 12 Empty the sample solution and rinse the beaker with distilled water.
- 13 Choose the next solution according to time available and repeat this procedure (B, steps 1 thru 12).

DATA TABLE
TITRATIONS TO COLOR END POINTS

	CASE 1 (OH) ⁻	CASE 2 (CO ₃) ⁼	CASE 3 (HCO ₃) ⁻	SAMPLE —
initial pH (Estimate)				
ml. @ M.O. End Pt.				
ml. @ P. End Pt.				
ml. @ Start				
TOTAL ml. HCl				
TOTAL ALKALINITY (mg CaCO ₃ /liter)				

VIII CALCULATIONS:

- A Calculate Total Alkalinity for each solution titrated, using the following:

$$\text{mg CaCO}_3 / \text{l} = \frac{A \times N \times 50,000}{\text{ml. of sample}}$$

A = total ml of standard acid required for titration to pH 4.5 end point

N = normality of standard acid

50 = equivalent weight of CaCO_3

1000 converts ml to liters

- B Record calculation results on the Data Tables.

REFERENCES

- 1 Methods for Chemical Analysis of Water and Wastes, EPA-AQCL, Cincinnati, OH 45268, (1971)
- 2 Standard Methods for the Examination of Water and Wastewater, 13th edition, APHA-AWWA-WPCF, Method 102 (1971)
- 3 ASTM Book of Standards, Part 23, p. 154 (1970)

This outline was prepared by A. Donahue, Chemist, National Training Center, DTTB, MDS, WPO, EPA, Cincinnati, OH 45268.

LABORATORY PROCEDURE FOR TOTAL ACIDITY

I REAGENTS

For detailed discussion of reagent preparation, consult reference 2.

A Carbon Dioxide - Free Distilled Water

B Potassium Hydrogen Phthalate
(primary standard)

C Sodium Hydroxide Titrant (0.02 N)

II STANDARDIZATION OF THE SODIUM HYDROXIDE TITRANT

A Set the temperature reading on the pH meter dial to match the temperature of the buffer and sample solutions.

B Standardize the pH meter against a reference buffer solution.

C Weigh accurately 0.19 ± 0.005 g of the dried potassium hydrogen phthalate and transfer it to a 500 ml conical flask.

D Add 100 ml of reagent water and swirl gently to dissolve the phthalate.

E While stirring the solution (magnetic bar and stirrer), add the sodium hydroxide from a 100 ml buret until a pH of 8.3 is attained.

F Calculate the normality of the sodium hydroxide solution as follows:

$$A = \frac{B}{0.20423XC}$$

A = normality of the sodium hydroxide

B = g of potassium hydrogen phthalate

C = ml of sodium hydroxide solution

0.20423 = milliequivalent weight of
potassium hydrogen phthalate
($\text{KHC}_8\text{H}_4\text{O}_4$)

III PROCEDURE

A Pipet 100 ml of the sample into a 400 ml beaker.

B Titrate with the sodium hydroxide to pH 8.3

C Calculation

Total acidity as mg of CaCO_3 /l =

$$\frac{A \times N \times 50000}{\text{ml of sample}}$$

A = ml of standard NaOH titrant

N = N of standard NaOH titrant

50,000 = equivalent weight of CaCO_3
in milligrams

REFERENCES

- 1 Methods for Chemical Analysis of Water & Wastes, EPA-AQCL, 1971, Cincinnati, OH 45268.
- 2 A.S.T.M. Book of Standards, Part 23, D 1067-70, pp. 155-158.

This outline was prepared by Charles R. Feldmann, Chemist, National Training Center, WPO, MDS, DTTB, EPA, Cincinnati, OH 45268.

LABORATORY PROCEDURE FOR ROUTINE LEVEL CHEMICAL OXYGEN DEMAND

I REAGENTS

A Standard Potassium Dichromate (0.250 N):

Dissolve 12.259 g of primary standard grade $K_2Cr_2O_7$, which had been dried at $103^\circ C$ for two hours, in distilled water and dilute to one liter.

B Ferrous Ammonium Sulfate (0.1N):

Dissolve 39 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water. Carefully add 20 ml of concentrated H_2SO_4 . Cool and dilute to one liter.

C Ferroin Indicator:

Dissolve 1.485 g 1, 10-phenanthroline monohydrate and 0.695 g $FeSO_4 \cdot 7H_2O$ in water and dilute to 100 ml. This indicator may be purchased already prepared.

D Concentrated Sulfuric Acid (36 N):

E Mercuric Sulfate: Analytical Grade

F Silver Sulfate: Analytical Grade

G Concentrated Sulfuric Acid - Silver Sulfate:

Dissolve 22 g of silver sulfate in a 9 lb bottle of concentrated sulfuric acid. (1-2 days required for dissolution)

II EQUIPMENT PREPARATION

Before use, the Erlenmeyer flask (500 ml, 24/40 standard taper joint) and reflux condenser (Friedrichs, 24/40 standard taper joint) should be steamed out to remove trace organic contaminants. Add 10 ml of 0.250 N $K_2Cr_2O_7$, 50 ml of distilled water, and several bumping stones to the flask. Carefully add 20 ml of concentrated H_2SO_4 and mix thoroughly. Connect the flask and condenser, but do not turn on the water to the condenser. Boil the mixture so that

steam emerges from the top of the condenser for several minutes. Cool the mixture, carefully discard the acid, and rinse the condenser and flask with distilled water. In order to prevent contamination from air-borne particles, the top of the condenser should be lightly plugged with glass wool during storage and use.

III STANDARDIZATION OF FERROUS AMMONIUM SULFATE

A Dilute 10.0 ml of the standard potassium dichromate to about 100 ml with distilled water.

B Add 30 ml of concentrated H_2SO_4 and allow to cool.

C Add 2-3 drops of ferroin indicator and titrate to a reddish-brown end point with the ferrous ammonium sulfate. Calculate the normality, N, of the ferrous ammonium sulfate.

D Calculation

$$N \text{ of } Fe(NH_4)_2(SO_4)_2 = \frac{\text{ml } K_2Cr_2O_7 \times N \text{ of } K_2Cr_2O_7}{\text{ml } Fe(NH_4)_2(SO_4)_2}$$

IV PROCEDURE

A Place 0.4 g $HgSO_4$ in a 500 ml 24/40 standard taper Erlenmeyer flask. Add 20 ml of sample, or an aliquot diluted to 20 ml with distilled water.

B Add 2 ml of concentrated H_2SO_4 and swirl to dissolve the $HgSO_4$.

Laboratory Procedure for Routine Level Chemical Oxygen Demand

- C Add 10.0 ml of the 0.250 N $K_2Cr_2O_7$ and mix.
- D Carefully add 28 ml of the sulfuric acid - silver sulfate reagent.
- E Add several pumice granules or glass beads to prevent bumping, and then swirl the mixture to insure complete mixing.
- F Reflux the mixture for two hours.
- G Allow the solution to cool, wash down the condenser with distilled water, and add about 75 ml water to bring the volume to about 150 ml.
- H Add 2-3 drops of the ferroin indicator and titrate the solution to a reddish-brown end point with the ferrous ammonium sulfate.
- I A blank consisting of 20 ml of distilled water and containing all reagents is refluxed and titrated in the same manner as the sample.

J Calculation

$$\text{mg COD/l} = \frac{(A-B) N \times 8 \times 1000}{\text{ml of sample}}$$

COD = chemical oxygen demand

A = ml $Fe(NH_4)_2(SO_4)_2$ used for blank

B = ml $Fe(NH_4)_2(SO_4)_2$ used for sample

N = N of $Fe(NH_4)_2(SO_4)_2$

8 = equivalent weight of oxygen

ACKNOWLEDGEMENT:

Portions of this outline were taken from an outline prepared by R. J. Liska.

REFERENCES

1. Methods for Chemical Analysis of Water & Wastes, EPA-AQCL, 1971.
2. Standard Methods, APHA-AWWA-WPCF, 13th Edition, 1971.
3. ASTM Standards, Part 23, 1970.

This outline was prepared by Charles R. Feldmann, Chemist, National Training Center, MDS, WPO, EPA, Cincinnati, OH 45268.

LABORATORY PROCEDURE FOR NITRATE NITROGEN
MODIFIED BRUCINE METHOD

(Range 0.1 to 2 mg nitrate nitrogen/l)

I REAGENTS

A Water

Distilled water free of nitrite and nitrate should be used in the preparation of all reagents and standards.

B Stock Potassium Nitrate
(1.0 ml = 0.1 mg NO_3^- N):

Dissolve 0.7218 g of anhydrous KNO_3 in distilled water and dilute to 1 liter.

C Standard Potassium Nitrate
(1.0 ml = 0.001 mg NO_3^- N):

Dilute 10.0 ml of the stock solution to 1 liter with distilled water. This solution should be prepared fresh weekly.

D Sulfuric Acid:

Carefully add 500 ml of concentrated H_2SO_4 (sp. gr. 1.84) to 125 ml of distilled water. Cool and keep tightly stoppered.

E Sodium Chloride (30%):

Dissolve 30 g of NaCl in distilled water and dilute to 100 ml.

F Brucine - sulfanilic acid reagent:

Dissolve 1 g of brucine sulfate, $(\text{C}_{23}\text{H}_{26}\text{N}_2\text{O}_4)_2 \cdot \text{H}_2\text{SO}_4 \cdot 7 \text{H}_2\text{O}$, and 0.1 g of sulfanilic acid ($\text{NH}_2\text{C}_6\text{H}_4\text{SO}_3\text{H} \cdot \text{H}_2\text{O}$) in 70 ml of hot distilled water. Add 3 ml of concentrated HCl, cool, and dilute to 100 ml. This solution is stable for several months if stored in a dark bottle at 5°C. The pink color which develops slowly does not effect the usefulness of the solution. The bottle should be marked "toxic".

G Acetic Acid:

Dilute 1 vol of glacial $\text{HC}_2\text{H}_3\text{O}_2$ with 3 vols of distilled water.

II SAMPLE PREPARATION

A In the case of highly alkaline waters, it is necessary to adjust the pH to approximately 7 with the acetic acid.

B Filter through a 0.45 micron pore size filter if necessary.

III PROCEDURE

A Pipet 0.0, 2.0, 5.0, 7.0, and 10.0 ml of the standard KNO_3 solution into 50 ml test tubes held in a suitable rack. Add sufficient distilled water to bring the volume to 10.0 ml.

B Pipet 2.0, 3.0, and 5.0 ml. of the sample into 50 ml test tubes. The purpose of using three different volumes of the sample is to ensure that when the colors are developed, at least one of the three samples will give an absorbance which lies within the range of the calibration curve. Add sufficient distilled water to bring the volume to 10 ml.

C If the samples are saline, pipet 2.0 ml of 30% NaCl into the standard and sample tubes. Mix well. This addition is unnecessary for fresh water samples.

D Chill all of the tubes to 0 - 10°C in a cold water bath.

E Pipet 10.0 ml of the H_2SO_4 solution into each standard and sample tube, mix well by swirling, and again chill to 0 - 10°C.

- F Pipet 0.5 ml of the brucine - sulfanilic acid reagent into the standard and sample tubes, mix well by swirling, and place the tubes in a boiling water bath for 25 minutes.
- G Remove the tubes from the boiling water bath and cool to 20-25°C in a water bath.
- H If color or turbidity develops in the sample while the tubes are in the boiling water bath, a blank must be prepared using all reagents except the brucine - sulfanilic acid.
- I Measure the absorbance values of the standards and samples at 410 nm. on a suitable spectrophotometer. Before each solution is read, rinse the spectrophotometer cell at least twice with the solution.
- J Prepare a calibration curve of absorbance values for the standards vs. mg of $\text{NO}_3^- \text{N}$. For example: if 2.0 ml of the standard KNO_3 solution are used, and its concentration is 0.001 mg of $\text{NO}_3^- \text{N/ml}$, then 0.002 mg of NO_3^- is the value plotted on the calibration curve vs. the corresponding absorbance value.

- K Determine the mg of $\text{NO}_3^- \text{N}$ present in the samples using the calibration curve and then calculate the mg of $\text{NO}_3^- \text{N/l}$ using the formula:

$$\text{mg of } \text{NO}_3^- \text{N/l} =$$

$$\text{mg of } \text{NO}_3^- \text{N from curve} \times \frac{1000 \text{ ml/l}}{\text{ml of sample}}$$

REFERENCES

Methods for Chemical Analysis of Water & Wastes, EPA-AQCL, 1971.

This outline was prepared by Charles R. Feldmann, Chemist, National Training Center, MDS, WPO, EPA, Cincinnati, OH 45268.

BIOCHEMICAL OXYGEN DEMAND TEST DILUTION TECHNIQUE

- I OBJECTIVES: to prepare a sample dilution for series observation of oxygen depletion versus time for the estimation of BOD_t, rate and ultimate demand. The azide modification of the Winkler iodometric titration of dissolved oxygen will be used. An electronic DO analyzer will be used to check the titrimetric DO.

II PROCEDURE:

- A Each group will prepare 1 dilution of either effluent or influent at an assigned concentration to enable filling 8 standard BOD bottles. One of these will be used for electronic DO monitoring by the lab instructor, the other seven are for daily student determinations. Identify each bottle with group number, I for influent or E for effluent and the assigned concentration in %.

- B Calculate the volume of sample to be used to make up a total diluted volume of 3500 ml at the assigned concentration. Then dilute the sample as follows:

- 1 Add dilution water to the dilution bottle to make up about 1/2 of the total amount of distilled water to be used. Add 1 ml each of the four mineral supplements. (These solutions are prepared at 4 times the concentration of Standard Methods mineral supplement reagents so the added volume is lower). Mix the supplemented dilution water thoroughly.
- 2 Carefully mix the bottle of sample to be used to a uniform suspension. If large chunks are evident, homogenize. Rapidly pour out, with mixing, into a beaker, the approximate amount of sample required and return to lab station. Rapidly measure out, with mixing, the required sample aliquot to make the 3500 ml sample dilution.

Mixing, time, and precision are important. Your sample may be non-representative to the extent of carelessness in mixing, allowing settling while adjusting measurement or measurement errors.

Add the measured quantity of sample to the dilution bottle.

- 3 Carefully add dilution water to make up 3500 ml of diluted sample.
 - 4 Stopper and shake the dilution bottle to mix the sample and to ensure that you have an acceptable initial DO. This step will add DO or reduce supersaturation. About 15 seconds of mixing is usually adequate.
- C Filling bottles is simplified if you place the dilution bottle on the center pedestal, insert the siphon and line up your identified bottles with stoppers removed. Have a container larger than the bottles available to accommodate the overflow while filling them. Otherwise, move the apparatus near a sink. Fill the siphon tube and waste some of the diluted sample to rinse this tube. Be sure that the siphon tube is used in a manner so that it is always full of the diluted sample. Agitate the diluted sample regularly to avoid undue collection of solids on the bottom.

Biochemical Oxygen Demand Test Dilution Technique

- 1 Place first bottle to be filled in the overflow container or hold over sink. Insert the siphon delivery tip all of the way down into the bottom of the test bottle. Tilt the bottle to cover the delivery tip with water promptly. Start the siphon flow slowly to decrease turbulence until the delivery tip is well-covered. Fill to overflowing. Slowly raise the delivery tip while overflowing the sample. Stop the flow with the bottle completely full. Insert the bottle stopper and turn it slightly to seat it. Fill the remaining bottles in the same manner.
- 2 Each bottle will have the same DO and sample mix as the next if you have followed a consistent procedure, mixed carefully, and have not changed technique enroute.
- 3 One of the eight bottles is to be retained for initial titration of DO by your group. Another bottle is to be turned in to the lab instructor for use in performance checks on all class dilutions. The remainder are to be placed into trays for 20° incubation. Be certain that all bottles are properly identified.

D Dissolved Oxygen Determination

- 1 You are encouraged to check the DO of your sample on the electronic analyzer. A calibrated unit will be available with assistance. The DO analyzer will not result in more than 1/2 ml loss of sample on probe insertion. You will be able to add DO reagents and continue the Winkler titrimetric DO on the same bottle.
- 2 An initial DO value (DO_0) using the Winkler method must be determined today. On succeeding days, the DO_t will be determined on the incubated samples for estimation of BOD progression. (If reaeration is required, the technique will be demonstrated). All data is to be recorded on the sheet provided in this manual.

The Winkler-Azide procedure as presented in outline 44 is to be used for these DO determinations.

- 3 A blank value for each stock bottle of distilled water will be determined by the staff.

This outline was prepared by F. J. Ludzack,
Chemist, National Training Center, DTTB,
MDS, WPO, EPA, Cincinnati, OH 45268.

Biochemical Oxygen Demand Test Dilution Technique

BOD DATA SHEET

Group No. _____

Initial Date _____

Buret Readings	DO _o	Buret Readings	DO _t	ΔDO	Sf/ID	BOD

Buret readings - initial and final

DO_o - initial DO in mg/liter

DO_t = DO at indicated incubation time_t

ΔDO = DO change (DO_o - DO_t); a depletion

Sf = the sample content as a decimal fraction in the dilution

ID = sample identification (influent or effluent)

BOD = $\frac{DO_o - DO_t}{Sf}$ (according to Standard Methods, 13th edition, 1971)

LABORATORY PROCEDURE FOR DISSOLVED OXYGEN

Winkler-Azide Procedure

I INTRODUCTION

The azide modification is used for most sewage, effluents, and streams which contain nitrate nitrogen and not more than 1 mg of ferrous iron/l. If 1 ml 40% KF solution is added before acidifying the sample and there is no delay in titration, the method is also applicable in the presence of 100-200 mg/l ferric iron.

The "Methods for Chemical Analysis of Water & Wastes, 1971," published by the Analytical Quality Control Laboratory of the EPA, recommends the Winkler-Azide method using the full bottle technique.

II REAGENTS

A Manganous Sulfate Solution

Dissolve 480 g $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ in distilled water and dilute to 1 liter.

B Alkali-Iodide-Azide Reagent

Dissolve 500 g sodium hydroxide and 150 g potassium iodide in distilled water and dilute to 1 liter. To this solution add 10 g sodium azide. NaN_3 dissolved in 40 ml distilled water.

C Sulfuric Acid, Conc.

The strength of this acid is 36 N.

D Starch Solution

Prepare an emulsion of 10 g of soluble starch in a mortar or beaker with a small quantity of distilled water. Pour this emulsion into 1 liter of boiling water, allow to boil a few minutes, and let settle overnight. Use the clear supernate. This solution may be preserved by the addition of 5 ml per liter of chloroform and storage in a 10°C refrigerator.

E Sodium Thiosulfate Stock Solution 0.75 N

Dissolve 186.15 g $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ in boiled and cooled distilled water and dilute to 1 liter. Preserve by adding 5 ml chloroform.

F Sodium Thiosulfate Standard Titrant 0.0375N

Dilute 50.0 ml of stock solution to 1 liter. Preserve by adding 5 ml of chloroform. Standardize with potassium biiodate.

III PROCEDURE

A Addition of Reagents

1 Manganous sulfate and alkali-iodide-azide

To a full sample bottle (300 ml + 3 ml BOD incubation), add 2 ml manganous sulfate solution and 2 ml alkaline-iodide reagent with the tip of each pipette below the surface of the sample.

2 Replace the stopper, rinse under running water and mix by inverting 4-5 times.

3 Allow the precipitate to settle until at least 100 ml of clear supernate have been produced. Repeat the inverting and settling.

4 Add 2 ml conc. sulfuric acid with the tip of the pipette above the surface of the sample.

5 Stopper the bottle, rinse under running water and mix to dissolve the precipitate.

6 Pour contents of bottle into a wide mouth 500 ml Erlenmeyer flask.

B Titration and Calculation

1 Titrate with 0.0375 N thiosulfate to a pale straw color, add 2 ml starch solution indicator and continue titrating to the disappearance of the blue color.

Laboratory Procedure for Dissolved Oxygen

2 Calculation

$$\frac{\text{mls titrant} \times 0.0375 \times 8000}{300} = \text{mg/l DO}$$

$$\text{mls titrant} \times 0.0125 \times 80 = \text{mg/l DO}$$

$$\text{mls titrant} \times 1 = \text{mg/l DO}$$

$$\text{mls titrant} = \text{mg/l DO}$$

REFERENCE

Methods for Chemical Analysis of Water and Wastes, EPA-AQCL, Cincinnati, Ohio, July 1971.

This outline was prepared by J. W. Mandia, Chemist, formerly with National Training Center, and revised by C. R. Feldmann, Chemist, National Training Center, MDS, WPO, EPA, Cincinnati, OH 45268.

LABORATORY PROCEDURE FOR CHLORINE RESIDUAL
Tap Water and Chlorinated Effluent Samples

A Hellige Color Comparator

1. Fill one sample tube with 10 ml of sample. Rinse tube 2-3 times with sample to be tested.
2. Add 1/2 ml of o-tolidine reagent mix and insert into the right or central tube position of the comparator.
3. Fill the second tube with sample to be tested. If the sample is turbid, add 1/2 ml of clear water, mix and insert into the left hand tube position of the comparator.
4. While looking through the comparator at a north light or equivalent, match the two color tubes by manipulation of the color discs on the wheel provided.
5. Read chlorine residual as indicated in the comparator window. Maximum color for free chlorine is almost instant; for combined chlorine, within 5 minutes.
6. If the color cannot be matched, use an aliquot of sample diluted to 10 ml with chlorine demand free water.

B Amperometric Titration

1. Fill the buret with phenylarsene oxide by carefully squeezing the reagent bottle. Displace air bubbles by overfilling the buret. Zero by turning the red knob on the titrator to the left about 1/4 turn. Be sure that the titrant delivery tube is inside of the cup, below the surface, and does not contain air bubbles. Take it easy with the red knob.
2. Fill the sample cup with sample to be tested to the 200 ml mark (or aliquot diluted to 200 ml with chlorine demand free water).
3. Insert in position on the titrator center post with the top of the cup behind the cup guide post, bottom resting on the two prongs in the slots provided and titrant delivery tube inside of the cup and about 1/16 inch below the water surface.
4. Rinse the cup and electrodes 2-3 times with sample to be tested by operating the motor 5 to 10 seconds for each rinse and refilling the cup each time with fresh sample.
5. Add 1 ml pH 7 buffer (not necessary if sample has a pH of 6.0 to 7.5).
6. Start agitator.
7. Adjust the potentiometer needle to on-scale position at the high reading position with the knob at the left of the scale.

Laboratory Procedure for Chlorine Residual

8. Add a small increment of phenyl arsene oxide (PAO). Presence of free chlorine will be indicated by a downward movement of the needle with each small increment of PAO. PAO addition without needle deflection indicates that you have no free chlorine or the endpoint has been exceeded. The needle movement must be on scale before each addition of PAO. Adjust with the control knob as necessary.
9. Add 1 ml of pH 4 buffer + 1 ml of KI solution and repeat the titration to measure total available chlorine residual.
10. The endpoint is indicated when the pointer will not move down-scale with titrant addition. Chlorine residual is numerically equal to volume of titrant added when 200 ml of sample has been used and the last addition of titrant (no needle deflection) has been subtracted from the total.
11. The electrode must be sensitized to iodine for total residual available chlorine determination. This is accomplished by allowing the electrode to soak in a solution containing Iodine + KI sufficient to produce a slight color for a minimum of 30 minutes. You may keep it that way by regular iodine titration or addition of iodine after you make the last titration of the day.

This outline was prepared by F. J. Ludzack,
Chemist, National Training Center, MDS, WPO,
EPA, Cincinnati, OH 45268.

SPECIFIC CONDUCTIVITY LABORATORY

I EQUIPMENT AND REAGENTS

A Equipment

- 1 Solu Bridge conductivity meters
- 2 Probes
 - a Cell VSO2
 - b Cell VS2
 - c Cell VS2O
- 3 Thermometers
- 4 400 ml beakers

B Reagents

- 1 Standard KCl solutions

Normality of KCl Solution	Specific Conductance micromhos/cm.
0.0001	14.9
0.001	147.0
0.01	1413.0
0.1	12900.0

- 2 Distilled water

II CHECKING THE INSTRUMENT

- A The measurement of specific conductivity as presented in sections II and III is written for one type of conductivity meter and probe.
- B A battery check is made by depressing the Battery Check switch, and at the same time pressing the on-off button. The meter needle should deflect to the right (positive) and come to rest in the green zone.
- C Place a 10,000 ohm resistor in the holes of the electrical contacts on the meter. Turn the temperature knob to read room temperature. Depress the on-off button and bring the meter needle to a reading

of 0 by turning the specific conductance switch. The specific conductance reading should be approximately 200 micromhos/cm.

III DETERMINATION OF THE CALIBRATION CONSTANT

- A Move the temperature switch so that its reading is the same as the temperature of the standard KCl and sample solutions.
- B Connect probe Cell VSO2 to the conductivity meter.
- C Rinse the probe in the beaker of distilled water, wipe the excess water with a kimwipe and place probe in the first beaker of KCl solution (0.0001 N).
- D Make certain the cell is submerged to a point at least 1/2 inch above the air hole and that no entrapped air remains. The cell should also be at least 1/2 inch from the inside walls of the flask.
- E Press and hold down the ON-OFF button, simultaneously rotating the main scale knob until the meter reads zero. Release the button. (If the meter needle remains off scale or cannot be nulled, discontinue testing in that solution.)
- F Record the scale reading in Table 1 and proceed to KCl solutions 0.001N, 0.01N, 0.1 N.
- G Repeat steps C through F using the VS2 and VS2O probes.
- H Compute the cell calibration constant-a factor by which scale readings must be multiplied to compute specific conductance:

$$K_{sp} = cM$$
 where K_{sp} = actual specific conductance,
 c = calibration constant
 M = meter reading
 (continued next page)

Specific Conductivity Laboratory

TABLE 1 DATA FOR CALIBRATION CONSTANTS

Probe	Cell VSO2				Cell VS2				Cell VS20			
KCl Solutions	0.0001N	0.001N	0.01N	0.1N	0.0001N	0.001N	0.01N	0.1N	0.0001N	0.001N	0.01N	0.1N
Test #												
1												
2												
3												
Cell Constant												

See I B Reagents for the actual specific conductance values of the KCl solutions used for the probes.

Record the computed cell constants on Table 1.

IV DETERMINATION OF THE SPECIFIC CONDUCTANCES OF THE SAMPLES

Determine the K_{sp} values of samples A, B and C using the procedure as described in Section III, steps C through F. Record data in Table 2.

V EPA METHODOLOGY

The current EPA Manual⁽¹⁾ specifies using the procedures found in References 2 and 3. These procedures have been adapted for this laboratory session.

ACKNOWLEDGMENT

This outline contains certain portions of previous outlines by Messrs. J. W. Mandia, and J. R. Tilstra.

REFERENCES

- 1 Methods for Chemical Analysis of Water and Wastes, EPA-AQCL, Cincinnati, OH 45268, 1971.
- 2 Standard Methods for the Examination of Water and Wastewater, 13th Edition. 1971.
- 3 Book of ASTM Standards, Part 23, 1970.

This outline was prepared by C. R. Feldmann, Chemist, National Training Center, and revised by Audrey E. Donahue, Chemist, National Training Center, DTTB, MDS, OWP, EPA, Cincinnati, OH 45268.

TABLE 2. SPECIFIC CONDUCTIVITY TESTS

Sample	A			B			C		
Probe	Cell VSO2	Cell VS2	Cell VS20	Cell VSO2	Cell VS2	Cell VS20	Cell VSO2	Cell VS2	Cell VS20
1									
2									
3									
Cell Constant									
Sp. Cond. μ mhos/cm									

LABORATORY PROCEDURE FOR PHOSPHORUS

I OBJECTIVE

The concentration of phosphorus (including available mineral, organic, poly and ortho phosphate) in the whole sample from a wastewater treatment plant is sought.

II METHOD SUMMARY:

- A The EPA Official Method is used. Certain non-official procedures are included to adapt this exercise for group participation and according to sample characteristics. Class use of 1 cm cells necessitates a stronger reference solution. Omitting the digestion of reference standards also deviates from the official method.
- B Mixed phosphorus forms in the sample will be converted into ortho phosphate by digestion at boiling temperature for 30 minutes in the presence of sulfuric acid and the oxidant, ammonium persulfate.

Excess acid will be neutralized, the sample diluted to its original volume and aliquots diluted to 50 ml for comparison with a series of reference sample dilutions and a reagent blank. Both sample and reference dilutions are adjusted to the same volume and mixed reagent added at the same time for comparative color development.

All solutions used in the procedure should be at room temperature or within $\pm 1^{\circ}\text{C}$ of the same temperature.

- C You may encounter 0.5 to 15 mg P/l in a sample of the type provided. Several small sample aliquots diluted to volume should be prepared along with your reference series to obtain at least one sample aliquot within the readable absorbance range of the references. Suggested sample aliquots in the procedure will give at least one readable result for a sample range of 0.1 to 20 mg P/liter.

III ANALYTICAL PROCEDURE

- A The conversion of phosphorus forms to orthophosphate

- 1 Measure 50 ml of the well mixed sample provided into one of the 125 ml erlenmeyer flasks.
- 2 Add one of the berl saddles to the sample aliquot.
- 3 Add one ml or 20 drops of strong acid to the sample aliquot.
- 4 Add 1 scoopful or approximately 0.4 g of dry powder ammonium persulfate, $(\text{NH}_4)_2\text{S}_2\text{O}_8$, to the sample aliquot.
- 5 Mix and place the sample flask on a preheated hot plate. Adjust heat to obtain a gentle boiling rate for 30 minutes. Add distilled water if necessary to prevent the sample volume from evaporating to a volume of less than 10 ml.
- 6 After 30 minutes boiling remove the digestion flask from the hot plate and cool it. If you cannot complete the analysis at this time, hold it at this stage after covering the flask with aluminum foil or an inverted beaker to protect it from contamination.

B Colorimetric Determination of Orthophosphate

- 1 Add 1 drop of phenolphthalein solution. Add NaOH solution dropwise to neutralize excess acid until the mixed digestate develops a red color. Add one drop of strong acid to eliminate the indicator color.
- 2 Adjust the volume of the digestate to its original volume of 50 ml by addition of distilled water. Mix.
- 3 Identify each of the seven remaining 125 ml erlenmeyer flasks as follows: Blank, 10-R, 20-R, 40-R, 0.5-S, 3.0-S and 10.0-S. (ml Reference solution-1.25 $\mu\text{g P/ml}$).
- 4 Add 50 ml of distilled water to the blank. Add 10 ml of reference solution + 40 ml of distilled water to the 10-R flask. Add appropriate amounts of reference solution or sample as indicated to the remaining flasks, plus enough distilled water to make a final volume of 50 ml in each flask. (It will not be necessary to add the indicator and adjust pH with NaOH or acid because all mixtures are approximately neutral at this stage. If you are not sure - check it).
- 5 Add 8 ml of mixed reagent to each of the reference and sample aliquots and to the 50 ml blank. Mix each one after addition.
- 6 After a 10 minute color development time (and not longer than 30 minutes), measure absorbancy of the color produced versus the reagent blank. (Adjust the instrument to zero absorbance (A) with the reagent blank in the light path. Use 880 nm wavelength). Record results on the following data table:

Item	ml R or S	$\mu\text{g P/50 ml}$	mg P/l	A
Blank	0	0	0	0
10-R	10-R	12.5	0.25	
20-R	20-R	25.0	0.50	
40-R	40-R	50.0	1.00	
0.5-S	0.5-S			
3.0-S	3.0-S			
10.0-S	10.0-S			

These data items require measurement of the absorbancy produced versus the reagent blank (adjust instrument to zero A for the blank) to estimate the mg P/l in the sample. Note that both the $\mu\text{g P/50 ml}$ and the equivalent mg P/l are listed in separate columns in the data record. The μg column is for check purposes to avoid calculation errors. Data is reported as mg P/liter.

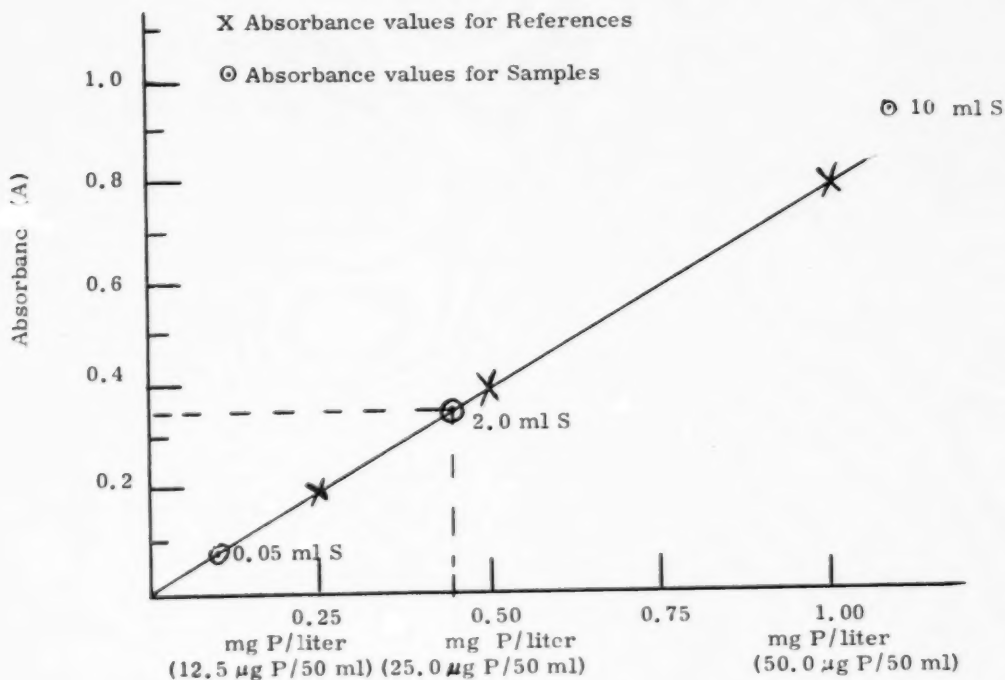
IV EVALUATING PHOSPHORUS CONCENTRATION

A Procedure

- 1 Consulting the data table for a range of values, mark absorbance vs mg P/liter scales on the coordinate paper provided. Also, mark the $\mu\text{g P}/50 \text{ ml}$ values below the mg P/liter scale values.
- 2 Plot the data for the Reference samples and draw a line of best fit.
- 3 Plot the data for the Sample aliquots and choose the valid result(s).
- 4 Evaluate the valid Sample points on the curve as mg P/liter.
- 5 Check calculations using the $\mu\text{g P}/50 \text{ ml}$ scale.
- 6 An example of this whole procedure follows.

B The Standard Curve

Reference and sample values have been plotted on this graph:



In this example, there is only one sample volume that falls on the most readable range of the instrument and within the calibrated range of the Absorbance plot - the 2.0 ml sample. The other two sample volumes gave invalid results.

- C Using the Standard Curve as shown in B above, the next step is to find mg P/liter of the sample when absorbance on the 2 ml sample diluted to 50 ml was 0.35: Draw a dashed horizontal line from the absorbance scale reading of 0.35 to the calibration line. Draw a vertical line from that intersection to the concentration scale. This reads approximately 0.45 mg P/liter for the diluted aliquot color. Since this sample was diluted from 2 to 50 ml, the original sample concentration was $0.45 \times \frac{50}{2} = 0.45 \times 25$ or 11.25 mg P/l.

D Checking Calculations:

With the same observed values and manipulation but using the $\mu\text{g P}/50\text{ ml}$ scale, the vertical intercept falls at about 22 $\mu\text{g P}/50\text{ ml}$ in the sample. For a sample containing 22 $\mu\text{g P}$ in the diluted 2 ml aliquot; the volume correction becomes $22 \times \frac{1000}{2} = 22 \times 500 = 11000\text{ }\mu\text{g P/l}$ or 11.00 mg P/l.

It is evident that the estimation of intercept positions on the small scales with infrequently marked positions yields a lack of precise agreement for the two independent calculations. The general agreement, however, does eliminate the question of a possible serious dilution error which frequently plagues the results.

This outline was prepared by F. J. Ludzack,
Chemist, National Training Center, DTTB,
MDS, WPQ EPA, Cincinnati, OH 45268.

ATOMIC ABSORPTION LABORATORY

ZINC ANALYSIS

Preparation of Standards:

A stock standard zinc solution was prepared by dissolving 1.000 grams of pure zinc metal in nitric acid and diluting to 1 liter with distilled water for a concentration of 1 ml = 1 mg Zn. Appropriate dilutions were made to provide 0, 0.1, 0.3, 0.5, 0.8, and 1.0 mg zinc/l standards.

Operating Conditions for P-E 303:

Wavelength	214
Range	UV
Slit	5
Source	10 ma, hollow cathode
Burner	P-E premix
Air	20 psi. (30 psi. on tank)
Auxiliary Air	9.0 on flowmeter
Acetylene	8 psi; 9.0 on flowmeter
Flame	clear and non-luminescent
Sample Uptake Rate	2-5 ml/min.

Zinc Analysis

ATOMIC ABSORPTION LABORATORY

Work Sheet

<u>mg Zn/l</u>	<u>% Absorption</u>	<u>Absorbance</u>
0.1	_____	_____
0.3	_____	_____
0.5	_____	_____
0.8	_____	_____
1.0	_____	_____

unknown

Concentration of unknown = _____ mg Zn/l

Scale expansion = _____

Suppression (meter response) = _____

This outline was prepared by R. J. Lishka,
Research Chemist, Bureau of Water
Hygiene, EPA, Cincinnati, OH 45268.

ABSORBANCE

ZINC CALIBRATION CURVE

DATE

0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
Concentration ZN Mg/Liter

ATOMIC ABSORPTION LABORATORY

COPPER ANALYSIS

Preparation of Standards:

A stock standard copper solution was prepared by dissolving 1.0000 gram of pure copper metal in nitric acid and diluting to 1 liter with distilled water for a concentration of 1 ml = 1 mg Cu. Appropriate dilutions were made to provide 0, 0.1, 0.3, 0.5, 0.8, and 1.0 mg copper/l standards.

Operating Conditions for P-E 303:

Wavelength	325
Range	UV
Slit	4
Source	15 ma., hollow cathode
Burner	P-E premix
Air	20 psi. (30 psi. on tank)
Auxiliary Air	9.0 on flowmeter
Acetylene	8 psi.; 9.0 on flowmeter
Flame	clear and non-luminescent
Sample Uptake Rate	2-5 ml/min.

ATOMIC ABSORPTION LABORATORY

Work Sheet

<u>mg Cu/l</u>	<u>% Absorption</u>	<u>Absorbance</u>
0.1	_____	_____
0.3	_____	_____
0.5	_____	_____
0.8	_____	_____
1.0	_____	_____
unknown	_____	_____

Concentration of unknown = _____ mg Cu/l

Scale expansion = _____

Suppression (meter response) = _____

This outline was prepared by R. J. Lishka,
Research Chemist, Bureau of Water
Hygiene, EPA, Cincinnati, OH 45268.

ABSORBANCE

0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

Concentration cu mg/ Liter

COPPER CALIBRATION CURVE

DATE

TABLE III - VALUES OF ABSORBANCE FOR PER CENT ABSORPTION

To convert per cent absorption (%A) to absorbance, find the per cent absorption to the nearest whole digit in the left-hand column; read across to the column located under the tenth of a per cent desired, and read the value of absorbance. The value of absorbance corresponding to 26.8% absorption is thus 0.1355.

%A	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
0.0	.0000	.0004	.0009	.0013	.0017	.0022	.0026	.0031	.0035	.0039
1.0	.0044	.0048	.0052	.0057	.0061	.0066	.0070	.0074	.0079	.0083
2.0	.0088	.0092	.0097	.0101	.0106	.0110	.0114	.0119	.0123	.0129
3.0	.0132	.0137	.0141	.0146	.0150	.0155	.0159	.0164	.0168	.0173
4.0	.0177	.0182	.0186	.0191	.0195	.0200	.0205	.0209	.0214	.0218
5.0	.0223	.0227	.0232	.0236	.0241	.0246	.0250	.0255	.0259	.0264
6.0	.0269	.0273	.0278	.0283	.0287	.0292	.0297	.0301	.0306	.0311
7.0	.0315	.0320	.0325	.0329	.0334	.0339	.0343	.0348	.0353	.0357
8.0	.0362	.0367	.0372	.0376	.0381	.0386	.0391	.0395	.0400	.0405
9.0	.0410	.0414	.0419	.0424	.0429	.0434	.0438	.0443	.0448	.0453
10.0	.0458	.0462	.0467	.0472	.0477	.0482	.0487	.0491	.0496	.0501
11.0	.0506	.0511	.0516	.0521	.0526	.0531	.0535	.0540	.0545	.0550
12.0	.0555	.0560	.0565	.0570	.0575	.0580	.0585	.0590	.0595	.0600
13.0	.0605	.0610	.0615	.0620	.0625	.0630	.0635	.0640	.0645	.0650
14.0	.0655	.0660	.0665	.0670	.0675	.0680	.0685	.0691	.0696	.0701
15.0	.0706	.0711	.0716	.0721	.0726	.0731	.0737	.0742	.0747	.0752
16.0	.0757	.0762	.0768	.0773	.0778	.0783	.0788	.0794	.0799	.0804
17.0	.0809	.0814	.0820	.0825	.0830	.0835	.0841	.0846	.0851	.0857
18.0	.0862	.0867	.0872	.0878	.0883	.0888	.0894	.0899	.0904	.0910
19.0	.0915	.0921	.0926	.0931	.0937	.0942	.0947	.0953	.0958	.0964
20.0	.0969	.0975	.0980	.0985	.0991	.0996	.1002	.1007	.1013	.1018
21.0	.1024	.1029	.1035	.1040	.1046	.1051	.1057	.1062	.1068	.1073
22.0	.1079	.1085	.1090	.1096	.1101	.1107	.1113	.1118	.1124	.1129
23.0	.1135	.1141	.1146	.1152	.1158	.1163	.1169	.1175	.1180	.1186
24.0	.1192	.1198	.1203	.1209	.1215	.1221	.1226	.1232	.1238	.1244
25.0	.1249	.1255	.1261	.1267	.1273	.1278	.1284	.1290	.1296	.1302
26.0	.1308	.1314	.1319	.1325	.1331	.1337	.1343	.1349	.1355	.1361
27.0	.1367	.1373	.1379	.1385	.1391	.1397	.1403	.1409	.1415	.1421
28.0	.1427	.1433	.1439	.1445	.1451	.1457	.1463	.1469	.1475	.1481
29.0	.1487	.1494	.1500	.1506	.1512	.1518	.1524	.1530	.1537	.1543
30.0	.1549	.1555	.1561	.1568	.1574	.1580	.1586	.1593	.1599	.1605
31.0	.1612	.1618	.1624	.1630	.1637	.1643	.1649	.1656	.1662	.1669
32.0	.1675	.1681	.1688	.1694	.1701	.1707	.1713	.1720	.1726	.1733
33.0	.1739	.1746	.1752	.1759	.1765	.1772	.1778	.1785	.1791	.1798
34.0	.1805	.1811	.1818	.1824	.1831	.1838	.1844	.1851	.1858	.1864
35.0	.1871	.1878	.1884	.1891	.1898	.1904	.1911	.1918	.1925	.1931
36.0	.1938	.1945	.1952	.1959	.1965	.1972	.1979	.1986	.1993	.2000
37.0	.2007	.2013	.2020	.2027	.2034	.2041	.2048	.2055	.2062	.2069
38.0	.2076	.2083	.2090	.2097	.2104	.2111	.2118	.2125	.2132	.2140
39.0	.2147	.2154	.2161	.2168	.2175	.2182	.2190	.2197	.2204	.2211
40.0	.2218	.2226	.2233	.2240	.2248	.2255	.2262	.2269	.2277	.2284
41.0	.2291	.2299	.2306	.2314	.2321	.2329	.2336	.2343	.2351	.2358

TABLE III CONTINUED

7A	0	1	2	3	4	5	6	7	8	9
42.0	.2366	.2373	.2381	.2388	.2396	.2403	.2411	.2418	.2426	.2434
43.0	.2441	.2449	.2457	.2464	.2472	.2480	.2487	.2495	.2503	.2510
44.0	.2518	.2526	.2534	.2541	.2549	.2557	.2565	.2573	.2581	.2588
45.0	.2596	.2604	.2612	.2620	.2628	.2636	.2644	.2652	.2660	.2668
46.0	.2676	.2684	.2692	.2700	.2708	.2716	.2725	.2733	.2741	.2749
47.0	.2757	.2765	.2774	.2782	.2790	.2798	.2807	.2815	.2823	.2832
48.0	.2840	.2848	.2857	.2865	.2874	.2882	.2890	.2899	.2907	.2916
49.0	.2924	.2933	.2941	.2950	.2958	.2967	.2976	.2984	.2993	.3002
50.0	.3010	.3019	.3028	.3036	.3045	.3054	.3063	.3072	.3080	.3089
51.0	.3098	.3107	.3116	.3125	.3134	.3143	.3152	.3161	.3170	.3179
52.0	.3188	.3197	.3206	.3215	.3224	.3233	.3242	.3251	.3261	.3270
53.0	.3279	.3288	.3298	.3307	.3316	.3325	.3335	.3344	.3354	.3363
54.0	.3372	.3382	.3391	.3401	.3410	.3420	.3429	.3439	.3449	.3458
55.0	.3468	.3478	.3487	.3497	.3507	.3516	.3526	.3536	.3546	.3556
56.0	.3565	.3575	.3585	.3595	.3605	.3615	.3625	.3635	.3645	.3655
57.0	.3665	.3675	.3686	.3696	.3706	.3716	.3726	.3737	.3747	.3757
58.0	.3768	.3778	.3788	.3799	.3809	.3820	.3830	.3840	.3851	.3862
59.0	.3872	.3883	.3893	.3904	.3915	.3925	.3936	.3947	.3958	.3969
60.0	.3979	.3990	.4001	.4012	.4023	.4034	.4045	.4056	.4067	.4078
61.0	.4089	.4101	.4112	.4123	.4134	.4145	.4157	.4168	.4179	.4191
62.0	.4202	.4214	.4225	.4237	.4248	.4260	.4271	.4283	.4295	.4306
63.0	.4318	.4330	.4342	.4353	.4365	.4377	.4389	.4401	.4413	.4425
64.0	.4437	.4449	.4461	.4473	.4485	.4498	.4510	.4522	.4535	.4547
65.0	.4559	.4572	.4584	.4597	.4609	.4622	.4634	.4647	.4660	.4672
66.0	.4685	.4698	.4711	.4724	.4737	.4750	.4763	.4776	.4789	.4802
67.0	.4815	.4828	.4841	.4855	.4868	.4881	.4895	.4908	.4921	.4935
68.0	.4948	.4962	.4976	.4989	.5003	.5017	.5031	.5045	.5058	.5072
69.0	.5086	.5100	.5114	.5129	.5143	.5157	.5171	.5186	.5200	.5214
70.0	.5229	.5243	.5258	.5272	.5287	.5302	.5317	.5331	.5346	.5361
71.0	.5376	.5391	.5406	.5421	.5436	.5452	.5467	.5482	.5497	.5513
72.0	.5528	.5544	.5560	.5575	.5591	.5607	.5622	.5638	.5654	.5670
73.0	.5686	.5702	.5719	.5735	.5751	.5768	.5784	.5800	.5817	.5834
74.0	.5850	.5867	.5884	.5901	.5918	.5935	.5952	.5969	.5986	.6003
75.0	.6021	.6038	.6055	.6073	.6091	.6108	.6126	.6144	.6162	.6180
76.0	.6198	.6216	.6234	.6253	.6271	.6289	.6308	.6326	.6345	.6364
77.0	.6383	.6402	.6421	.6440	.6459	.6478	.6498	.6517	.6536	.6556
78.0	.6576	.6596	.6615	.6635	.6655	.6676	.6696	.6716	.6737	.6757
79.0	.6778	.6799	.6819	.6840	.6861	.6882	.6904	.6925	.6946	.6968
80.0	.6990	.7011	.7033	.7055	.7077	.7100	.7122	.7144	.7167	.7190
81.0	.7212	.7235	.7258	.7282	.7305	.7328	.7352	.7375	.7399	.7423
82.0	.7447	.7471	.7496	.7520	.7545	.7570	.7595	.7620	.7645	.7670
83.0	.7696	.7721	.7747	.7773	.7799	.7825	.7852	.7878	.7905	.7932
84.0	.7959	.7986	.8013	.8041	.8069	.8097	.8125	.8153	.8182	.8210
85.0	.8239	.8268	.8297	.8327	.8356	.8386	.8416	.8447	.8477	.8508
86.0	.8539	.8570	.8601	.8633	.8665	.8697	.8729	.8761	.8794	.8827
87.0	.8861	.8894	.8928	.8962	.8996	.9031	.9066	.9101	.9136	.9172
88.0	.9208	.9245	.9281	.9318	.9355	.9393	.9431	.9469	.9508	.9547
89.0	.9586	.9626	.9666	.9706	.9747	.9788	.9830	.9872	.9914	.9957

FLAME PHOTOMETRY LABORATORY

SODIUM ANALYSIS, 0-10 mg/l, (Absolute Method)

Preparation of Standards:

A 1000 mg/l stock standard sodium solution was prepared by dissolving 2.5419 grams NaCl, dried by fusing at 900°C for 1/2 hour, in deionized distilled water. This was diluted to 1 liter for 1 ml = 1.000 mg Na. This stock standard was diluted 1:10 for a 1 ml = 0.1 mg Na standard solution. Appropriate dilutions were made to provide 0, 2, 4, 6, 8, and 10 mg Na/l standards.

Operating Conditions:

Theoretical wavelength 589 nm.

Wavelength used _____ nm.

Wavelength at which background measured _____ nm.

Instrument No. 1. Slit width _____ mm.

Fuel pressure 7.5 lbs/sq. in. O₂ pressure 10 lbs./sq. in.

Sensitivity selector on 4.

Sodium Analysis, 0-10 mg/l, (Absolute Method)

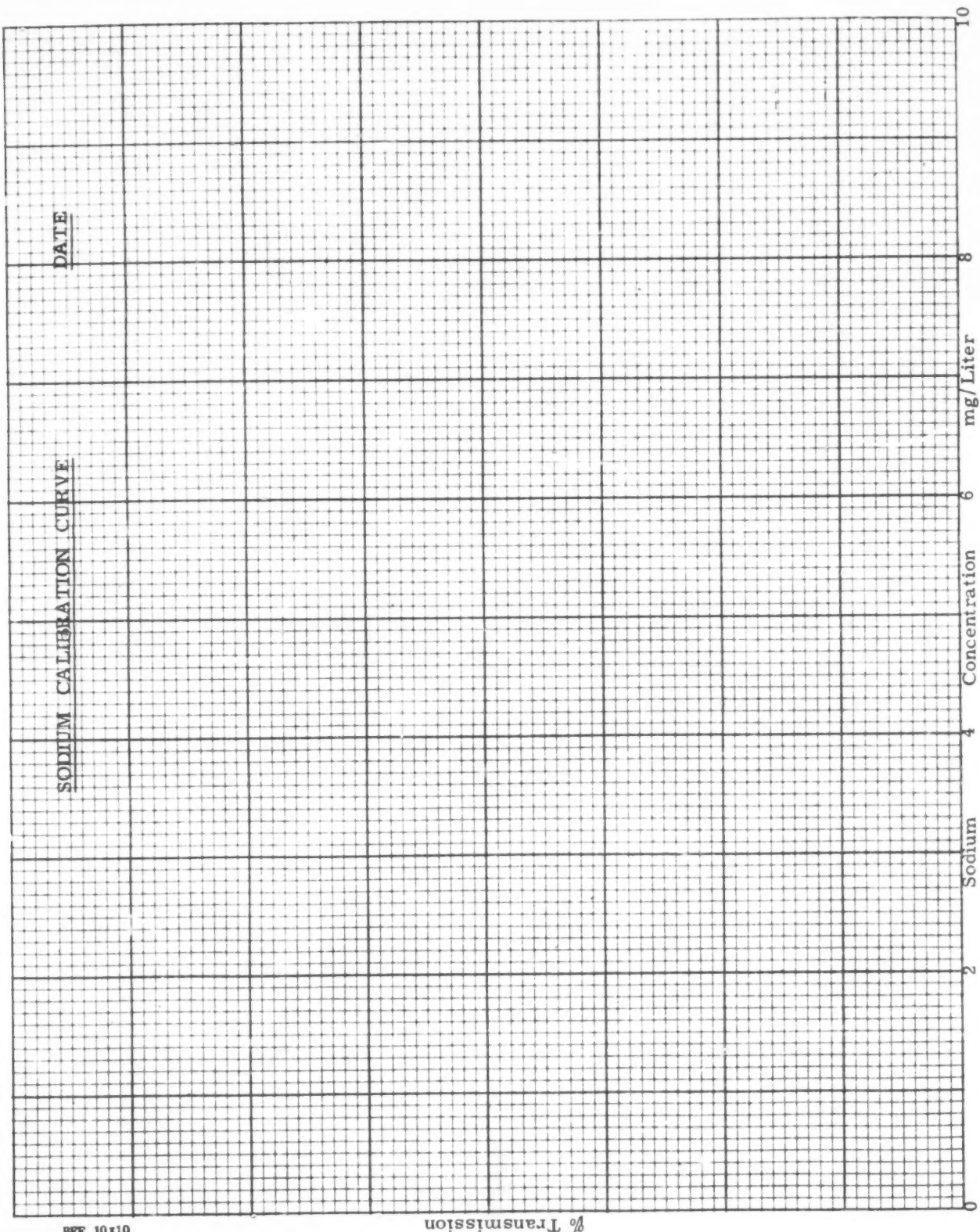
% TRANSMISSION READINGS:

	BACKGROUND	PEAK	DIFFERENCE
0 mg/l			
Avg.	_____	_____	_____
2 mg/l			
Avg.	_____	_____	_____
4 mg/l			
Avg.	_____	_____	_____
6 mg/l			
Avg.	_____	_____	_____
8 mg/l			
Avg.	_____	_____	_____
10 mg/l			
Avg.	_____	_____	_____

Subtract the background reading from the peak reading and plot the difference. The mg Na/l should be plotted along horizontal axis. versus the % transmission on vertical axis.

_____ mg Na/l

This outline was prepared by R. J. Lishka,
Chemist, Bureau of Water Hygiene,
EPA, Cincinnati, OH 45268.



DATE

SODIUM CALIBRATION CURVE

BSE 10x10

% Transmission

0 2 4 6 8 10
Sodium Concentration mg/Liter

SODIUM ANALYSIS

READ THE ENTIRE PROCEDURE BEFORE BEGINNING

1. Number the six plastic cups provided 0, 2, 4, 6, 8, and 10.
2. Fill them 3/4 full with the appropriate sodium standards; e.g., 0 mg/l standard into cup 0, etc.
3. Fill a 7th plastic cup 3/4 full with the unknown.
4. Fill an 8th plastic cup with distilled water.
5. The power toggle switch (on left side of instrument) is already turned on.
6. Set the sensitivity knob to the standby position.
7. Set the wavelength knob to the theoretical value of 589 nm (the scale is at the top of the instrument).
8. The fltr-shtr-open knob is in the shtr. (closed) position.
9. Open the main valve on the oxygen cylinder; all other oxygen gauges are already set.
10. Open the main valve on the hydrogen cylinder; all other hydrogen gauges are already set.
11. Raise the door on the right side of the burner housing (behind instrument).
12. Cautiously bring a lighted match to the tip of the burner in the housing.
13. Close the door on the burner housing.
14. Caution: Do not place any part of your body over the coils on the top of the burner housing. Hot gases are escaping.
15. Raise the silver lever between the instrument and the burner housing to the vertical position.
16. Place the plastic cup containing distilled water in the cup holder which is now exposed at the right side of the burner housing.
17. Push the silver lever clockwise so that the cup holder swings into the burner housing and the water is aspirated. A distinct difference in sound will be noticed when water or a sample is being aspirated. If at any time during the determination this sound again changes, it will indicate that all of the liquid has been aspirated from the cup. Simply move the silver lever and refill the cup with the appropriate liquid.
18. Do not allow air to be aspirated for more than about 15 seconds. If there is any delay, aspirate distilled water until the problem causing the delay has been corrected.
19. Turn the sensitivity knob to position 1.
20. Turn the dark current knob until the needle reads 0 on the percent transmittance scale.

21. Turn the sensitivity knob to position 4.
22. Repeat step 20.
23. Swing the cup of distilled water out of the burner housing and replace it with cup 10. Swing this cup back into the burner housing.
24. Turn the fltr - shtr - open knob to the open position (this opens the shutter).
25. Turn the wavelength knob slowly to the left; the needle will move to the left.
26. At some point the needle will suddenly swing toward the right. It will probably be necessary to make adjustments with the slit knob in order to keep the needle on-scale while finding the point at which the needle swings back to the right. Record this wavelength. It is the peak wavelength. Do not change this setting until indicated in the instructions.
27. If the point at which the needle swings back to the right is overshoot turn the wavelength knob about 1/4 turn to the right and repeat steps 25 and 26.
28. Make the needle read 100 percent transmittance by turning the slit knob. Record the slit mm reading. Do not change this setting. 100 is the record transmittance reading for this solution.
29. Turn the fltr - shtr - open knob to the shtr position (this closes the shutter).
30. Replace cup 10 with cup 8.
31. Open the shutter.
32. Record the percent transmittance reading and close the shutter.
33. Repeat steps 30, 31, and 32 using cups 6, 4, 2, 0, and the unknown, in turn.
34. Aspirate distilled water and using the dark current knob make the needle read 0 percent transmittance.
35. Aspirate cup 10.
36. Open the shutter.
37. Slowly turn the wavelength knob to the left. The needle will move to the right and at about 1/4- 1 percent transmittance will move no further to the right. Record the wavelength reading. This is the background wavelength. Do not change this setting. The percent transmittance is the background reading for this solution.
38. If the point at which the needle moves no further to the right is overshoot, turn the wavelength about 1/4 turn to the right and repeat step 37.
39. Close the shutter.

Sodium Analysis, 0-10 mg/l, (Absolute Method)

40. Replace cup 10 with cup 8.
41. Open the shutter.
42. Record the background transmittance reading for this solution.
43. Close the shutter.
44. Repeat steps 40, 41, 42 and 43 using cups 6, 4, 2, 0, and the unknown, in turn.
45. Aspirate distilled water for about 15 sec.
46. Turn the sensitivity knob to the standby position.
47. Close the main valve on the hydrogen cylinder.
48. Close the main valve on the oxygen cylinder.
49. Empty the eight plastic cups and discard them.
50. Leave the power toggle switch (on left side of instrument) on.
51. For each of the 6 solutions subtract the background percent transmittance reading from the peak percent transmittance reading.
52. Using the graph paper provided in the manual, plot the 6 differences vs. the appropriate concentrations. Draw the line of best fit connecting the 6 points. This is the calibration graph.
53. Find the difference percent transmittance for the unknown on the percent transmittance axis.
54. Draw a straight line to the right until it intersects the calibration line.
55. From the point of intersection draw a line straight down to the concentration axis.
56. This is the concentration of the unknown.

This procedure was prepared by C. R. Feldmann, Chemist, National Training Center, DTTB, MDS, WPQ EPA, Cincinnati, OH 45268.

FLAME PHOTOMETRY LABORATORY

STRONTIUM ANALYSIS (Standard Addition Method)

Preparation of Standards

A stock strontium solution, 1 ml = 1 mg Sr, was prepared by adding 1.685 g of SrCO_3 to 300 ml of deionized water, and dissolved by adding 6 ml of 1:1 HCl. The solution was boiled gently to expel CO_2 and diluted to 1 liter. A 1:10 dilution was made to obtain a 1 ml = 0.1 mg standard solution. Appropriate dilutions were made to provide 0, 5, 10, 15, and 20 mg Sr/l standards. One volume of "unknown" sample was added to one volume of each of the standards, including the zero standard.

Operating Conditions

Theoretical wavelength 461 nm

Wavelength used _____ nm

Wavelength at which background measured _____ nm

Instrument No. 3. Slit width _____ mm.

Fuel pressure 7.5 lbs/sq. in. O_2 pressure 10 lbs/sq. in.

Use photomultiplier "E" setting.

Sensitivity selector on 4.

Strontium Analysis (Standard Addition Method)

ANALYSIS OF UNKNOWN SOLUTION

% TRANSMISSION READINGS:

BACKGROUND	PEAK	DIFFERENCE
10 ml unknown + 10 ml 0 mg/l STD Avg.		
10 ml unknown + 10 ml 5 mg/l STD Avg.		
10 ml unknown + 10 ml 10 mg/l STD Avg.		
10 ml unknown + 10 ml 15 mg/l STD Avg.		
10 ml unknown + 10 ml 20 mg/l STD Avg.		

- (1) Subtract the background reading from the peak reading and plot the difference:
% transmission vs. concentration

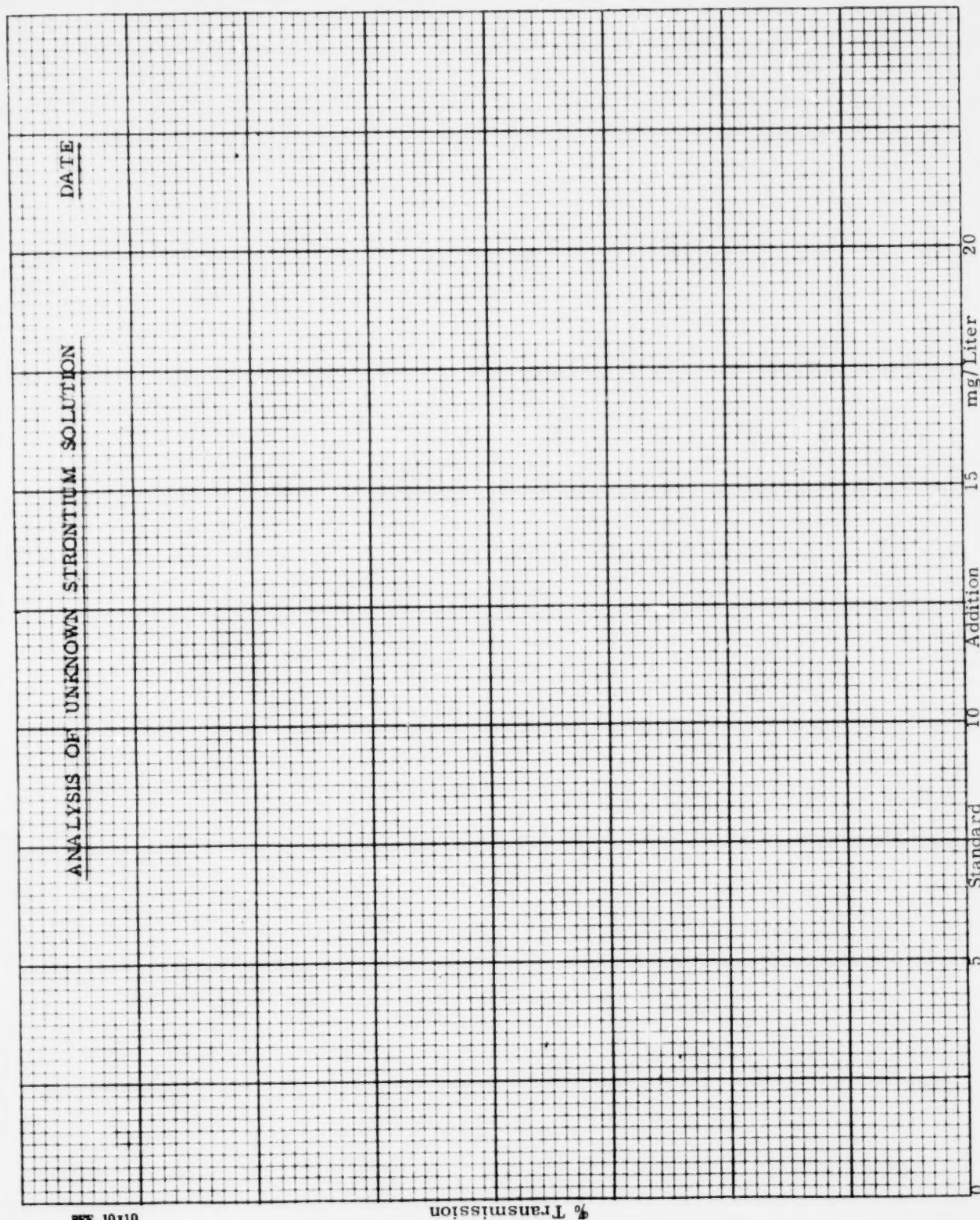
- (2) Double % transmission reading of mixture containing unknown sample and zero standard

% T

- (3) Determine concentration of unknown sample from calibration curve

Sr mg/l

This outline was prepared by R. J. Lishka,
Chemist, Bureau of Water Hygiene, EPA,
Cincinnati, OH 45268.



10x10 328

Strontium Analysis (Standard Addition Method)

READ THE ENTIRE PROCEDURE BEFORE BEGINNING

1. Number the five volumetric flask provided 0, 5, 10, 15, and 20.
2. Into each of the five, pipette 10.0 ml of the unknown.
3. Using a clean pipette, add 10.0 ml of the 0mg/l standard into flask 0.
4. Again using a clean pipette, add 10.0 ml of the 5 mg/l standard into flask 5.
5. Proceed in a similar manner using the 10, 15, and 20 mg/l standards and flasks 10, 15, and 20.
6. Stopper and shake all five flasks.
7. Mark five plastic cups 0, 5, 10, 15, and 20.
8. Fill the plastic cups about 3/4 full with the appropriate solutions from the volumetric flasks.
9. Fill a 6th plastic cup with distilled water.
10. The power toggle switch (on left side of instrument) is already turned on.
11. Set the sensitivity knob to the standby position.
12. Set the phototube voltage knob (on right side of instrument) to position E.
13. Set the wavelength knob to the theoretical value of 461 nm (the scale is at the top of the instrument).
14. Set the fltr -shtr -open knob in the shtr position (closed position).
15. Open the main valve on the oxygen cylinder; all other oxygen gauges are already set.
16. Open the main valve on the hydrogen cylinder; all other hydrogen gauges are already set.
17. Raise the door on the right side of the burner housing (behind instrument).
18. Cautiously bring a lighted match to the tip of the burner in the housing.
19. Close the door on the right side of the burner housing.
20. Caution: Do not place any part of your body over the coils on the top of the burner housing. Hot gases are escaping.
21. Raise the silver lever between the instrument and the burner housing to the vertical position.
22. Place the plastic cup containing distilled water in the cup holder which is now exposed at the right side of the burner housing.
23. Push the silver lever clockwise so that the cup holder swings into the burner housing and the water is aspirated. A distinct difference in sound will be noticed when water or a sample is being aspirated. If at any time during the determination this sound again changes, it will indicate that all of the liquid has been aspirated from the cup. Simply move the silver lever and refill the cup with the appropriate liquid.
24. Do not allow air to be aspirated for more than about 15 sec. If there is any delay, aspirate distilled water until the problem causing the delay has been corrected.
25. Turn the sensitivity knob to position 1.
26. Turn the dark current knob until the needle reads 0 on the percent transmittance scale.
27. Turn the sensitivity knob to position 3.
28. Repeat step 26.

29. Swing the cup of distilled water out of the burner housing and replace it with cup 20. Swing this cup back into the burner housing.
30. Turn the fltr -shtr -open knob to the open position (this opens the shutter).
31. Turn the wavelength knob slowly to the left; the needle will move to the left.
32. At some point the needle will suddenly swing toward the right. It will probably be necessary to make adjustments with the slit knob in order to keep the needle on-scale while finding the point at which the needle swings back to the right. Record this wavelength. It is the peak wavelength. Do not change this setting until indicated in the instructions.
33. If the point at which the needle swings back to the right is overshoot, turn the wavelength knob about 1/4 turn to the right and repeat steps 31 and 32.
34. Make the needle read 100 percent transmittance by turning the slit knob. Record the slit mm reading. Do not change this setting. 100 is the peak percent transmittance reading for this solution.
35. Turn the fltr - shtr -open knob to the shtr position (this closes the shutter).
36. Replace the cup 20 with cup 15.
37. Open the shutter.
38. Record the percent transmittance reading.
39. Close the shutter.
40. Repeat steps 36, 37, 38, and 39, using cups 10, 5, and 0 in turn.
41. Aspirate distilled water and using the dark current knob make the needle read 0 percent transmittance.
42. Aspirate cup 20.
43. Open the shutter.
44. Slowly turn the wavelength knob to the left. The needle will move to the right and at about 10-30 percent transmittance will move no farther to the right. Record the wavelength reading. This is the background wavelength. Do not change this setting. The percent transmittance is the background reading for this solution.
45. If the point at which the needle moves no farther to the right is overshoot, turn the wavelength about 1/4 turn to the right and repeat step 44.
46. Close the shutter.
47. Replace cup 20 with cup 15.
48. Open the shutter.
49. Record the background transmittance reading for this solution.
50. Close the shutter.

Strontium Analysis (Standard Addition Method)

51. Repeat steps 47, 48, 49, and 50 using cup 10, 5, and 0 in turn.
52. Aspirate distilled water for about 15 sec.
53. Turn the sensitivity knob to the standby position.
54. Close the main valve on the hydrogen cylinder.
55. Close the main valve on the oxygen cylinder.
56. Empty all six plastic cups and discard them.
57. Leave the power toggle switch (on left side of instrument) on.
58. For each of the 5 solutions, subtract the background percent transmittance reading from the peak percent transmittance reading.
59. Using the graph paper provided in the manual, plot the 5 differences vs. the appropriate concentrations. Draw a straight line connecting the five points. This is the calibration graph.
60. Double the difference value obtained for the solution in cup 0.
61. Find this value on the percent transmission axis.
62. Draw a straight line to the right until it intersects the calibration line.
63. From the point of intersection, draw a line straight down to the horizontal axis.
64. This is the concentration of the unknown.

This procedure was prepared by C. R. Feldmann, Chemist, National Training Center, MDS, DTTB, WPO, EPA, Cincinnati, OH 45268.

THIN LAYER CHROMATOGRAPHY LABORATORY

I PREPARATION OF PLATE

- A Check plate coating for uniformity. There should be a 1/4 inch scraped area on two opposite edges.
- B Place the plate on an open kimwipe on the bench top.
- C Place the template over the plate with the graduated edge about one inch from the bottom. Scrape a small mark on the plate at both ends of this spotting line.
- D Use template straight edge and score a line 10 cm away from and parallel to the spotting line. (This score stops movement of the solvent front).
- E Re-position the template so that spotting positions lay at marked spotting line.
- C Insert capillary tube into the sample, fill, and lightly touch tube tip to the desired spotting position on the plate. Remove about 1/4 of tube contents, allow to dry, spot another 1/4 of contents, dry and so on until capillary tube is drained.
- D Rinse capillary tube 5 times with hexane, as in "A".
- E Record the sample identification, volume spotted, and position number of the spot on data sheet.
- F Repeat steps B, C, D, and E, until all samples have been spotted on the plate.
- G Remove template.

II SETTING UP WORK AREA AND SAMPLES

- A Place folded kimwipe on left hand side of work area.
- B Place sample vials alongside of kimwipe.
- C A small beaker of reagent-grade hexane should also be placed near the sample vials.
- D Insert proper volume capillary tube in holder.

IV DEVELOPMENT IN SOLVENT

- A Place plate in developing tank containing specified solvent--spotting line down with coated surface facing the solvent saturated paper.
- B Let stand until solvent reaches scored line. Immediately remove the plate, taking care not to disturb the coating.
- C Allow plate to dry (2-5 minutes) and continue with Visualization as in next section.

III SPOTTING PROCEDURE

- A Insert capillary tube into hexane, fill, and touch to folded kimwipe until all hexane has drained from the capillary. Repeat this for 5 rinses of tube.
- B Insert capillary tube into desired sample, fill, touch to folded kimwipe until all contents are drained. Two such sample rinses are sufficient.

V VISUALIZATION

- A Chlorinated hydrocarbons - Al_2O_3 plates; solvent is 1% ethyl ether in hexane.
 - 1 After solvent treatment and drying, spray (in hood) with 0.01% Rhodamine B in 95% ethanol.
 - 2 After 5 minutes, observe spots with ultra violet light and use a paper clip to score the center of each spot.

VI MEASUREMENTS

- A Lay template over the coated plate.
 - B Using template or a cm ruler, measure and record the distance (cm) from the spotting (start) line to the center of each spot.
 - C On the data sheet, record the distance of the solvent front from the spotting (start) line to the finish line (pre-scored at 10 cm).
- B Inject 5 - 10 μ l of a 0.10 ng/ μ l solution of aldrin into the G. C.
 - C Compare the peak heights and determine the amount of aldrin recovered in this procedure.

VII CALCULATIONS

This outline was prepared by A. E. Donahue, Chemist, National Training Center, DTTB, MDS, WPO, EPA, Cincinnati, OH 45268.

- A $R_f = \frac{\text{distance in cm of sample from start}}{\text{distance in cm of solvent front from start}}$
- B $R_x = \frac{\text{distance in cm of sample from start}}{\text{distance in cm of reference compound from start}}$

VIII RECOVERY OF COMPONENT

- A Score a square around the 5 μ g aldrin spot.
- B Remove the scored area of aldrin- Al_2O_3 with the dropper collection assembly (glass wool in tapered end which is attached to a vacuum line).
- C Using a paper clip as a support, position the dropper with tapered end down inside the neck of a 50 ml. volumetric flask. Elute the aldrin into the flask using several rinses (about 5 ml. total) of 4:1 hexane-ether mixture. Remove the dropper.
- D Using hexane, bring the aldrin solution in the flask to 50.0 ml volume. Insert stopper.

IX DETERMINATION OF COMPONENT CONCENTRATION

- A Inject 5 - 10 μ l of the aldrin solution into a gas chromatograph with an electron capture detector.

PLATE COATING _____

Solvent (f) _____

Chromogenic Development _____

Reference Compound (x) _____

DATE _____

CLASS OF COMPOUND Chlorinated Hydrocarbons

SAMPLE	VOLUME (μ l) (1-2g/1 μ l)	SPOT POSITION	SAMPLE SPOT POSITION FROM START (cm)	SOLVENT FRONT DISTANCE FROM START (cm)	$R_f =$ SAMPLE DISTANCE SOLVENT DISTANCE	$R_x =$ SAMPLE DISTANCE REFERENCE COM- POUND DISTANCE
Aldrin	5					
Aldrin	10					
Dieldrin	5					
Dieldrin	10					
DDE	5					
DDT	5					
Lindane	5					
Lindane	10					
Parathion	5					
Parathion	10					

INFRARED
Procedure For Use Of Infracord

1. The "on-off" toggle switch should be in the "on" position, and the instrument warmed up for about 15 minutes.
2. The "reset-stop-scan" knob should be in the "reset" (extreme counter-clockwise) position; never try to manually move the chart drum unless this knob is in the "reset" position.
3. Please do not touch the "slit" or "100%" knobs, or those on the side of, or in the rear of, the instrument.
4. The pen holder assembly should be drawn back so that the pen is not touching the chart drum.
5. With the "reset-stop-scan" switch in the "reset" position, gently remove the chart drum from the instrument.
6. Place a piece of recorder paper on the drum in such a way that the guidelines above and below the hole in the upper left corner of the paper are aligned with the line inscribed in the drum itself. Replace the drum in the instrument.
7. Gently move the pen holder assembly forward so that the tip of the pen touches the paper.
8. Lay the two sodium chloride salt plates provided on a tissue. Always hold the sodium chloride salt plates with a tweezer or the finger tips; in the latter case, hold the plates by the ends, or narrow sides. Never hold the plates by the wide sides since these are the optical faces.
9. Dip the end of a capillary tube into the liquid sample to a depth of 1-2 mm. The liquid will rise in the tube via capillary action.
10. Holding the capillary tube containing the sample in a vertical position, touch it lightly several times to the center of a salt plate; a small spot of sample will be formed.
11. Place the second salt plate on top of the first plate and press down lightly; the spot should spread out so as to cover almost the entire area between the two plates.
12. Remove the spring clip holder from the instrument and place the two-plate "sandwich" in it.
13. Place the holder in the sample beam (front) bracket on the instrument.
14. Move the "reset-stop-scan" knob to the scan position; the rest of the process is automatic and the drum will stop revolving when the spectrum of the sample has been completed (about 13 minutes).

Infrared

15. Draw the pen holder assembly back from the chart paper.
16. Move the "reset-stop-scan" knob to the "reset" position and slowly rotate the drum in a counter clockwise direction until it stops.
17. Remove the drum from the instrument, and then, the chart paper from the drum.
18. Place a new piece of chart paper on the drum and place the drum back in the instrument.
19. Remove the spring clip holder from the instrument.
20. Remove the salt plat "sandwich" from the holder and separate the two plates.
21. Using a tissue dipped in the beaker of solvent, gently, but thoroughly, wipe the sample from both of the salt plates; allow the plates to air dry.

This outline has been prepared by Charles
R. Feldmann, Chemist, National Training
Center, MDS, WPO, EPA, Cincinnati,
OH 45268.